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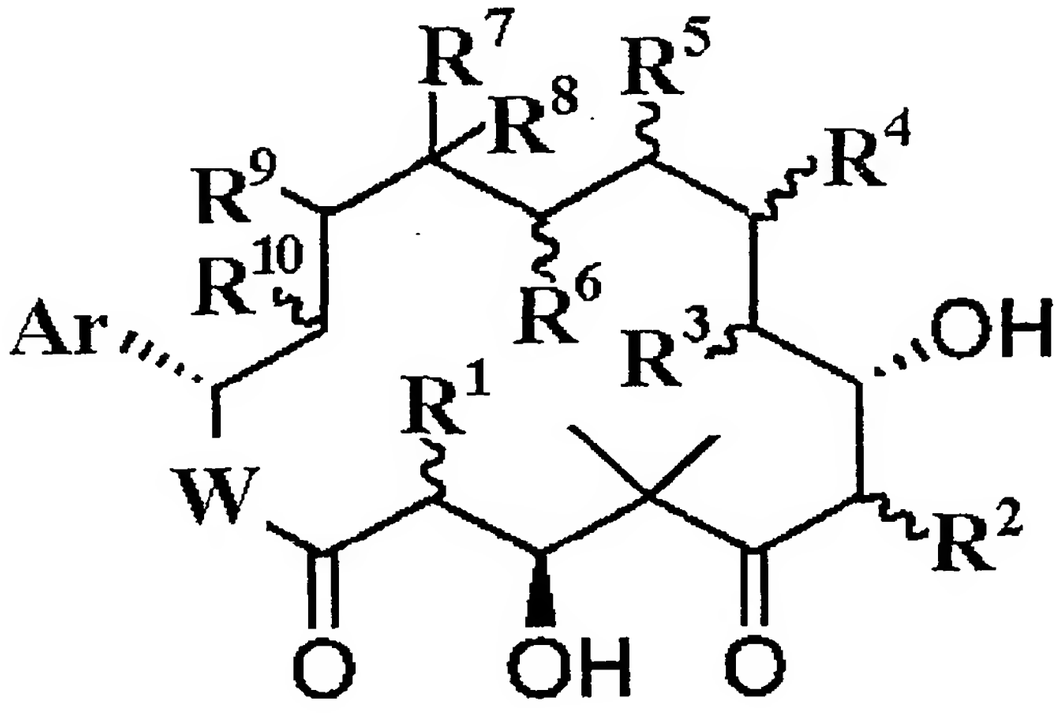
(54) Title: EPOTHILONE DERIVATIVES AND METHODS FOR MAKING AND USING THE SAME



(I)

(57) Abstract: The present invention relates to 16-membered macrocyclic compounds. In one aspect of the present invention, compounds of the formula (I), are provided wherein: R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl; R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl; R^5 is hydrogen, oxo, or C_1-C_{10} aliphatic, or optionally R^4 and R^5 together form a carbon-carbon double bond; R^6 is hydrogen, hydroxyl, oxo, C_1-C_{10} aliphatic, C_1-C_{10} alkylester, or halide; R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen, or optionally, R^6 and R^7 together form a 1,3-dioxane that is optionally substituted at the 2-position; R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide; Ar is aryl; and, W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl provided that at least one of R^4, R^5, and R^6 is not hydrogen. These compounds are cytotoxic agents and can be used to treat cancer and non-cancer disorders characterized by cellular hyperproliferation.

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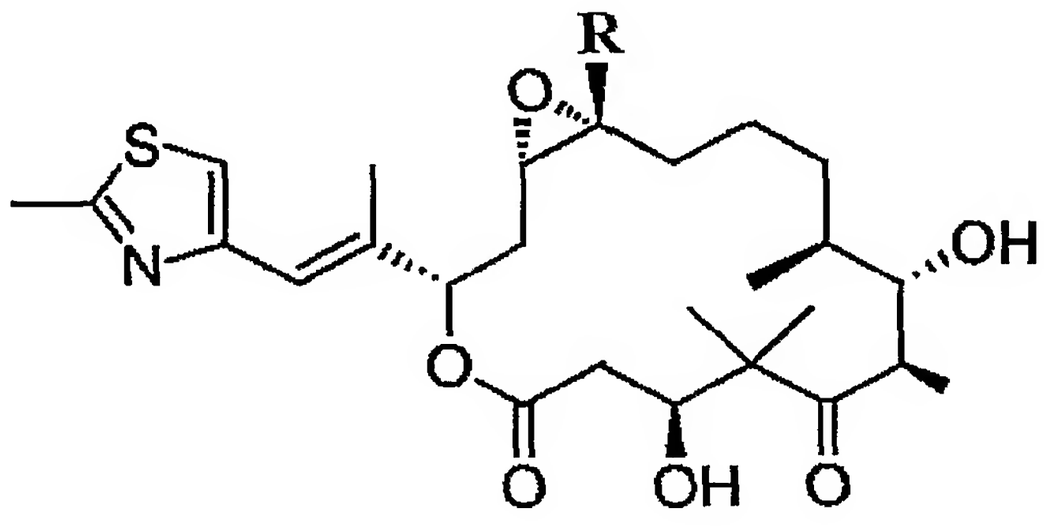
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

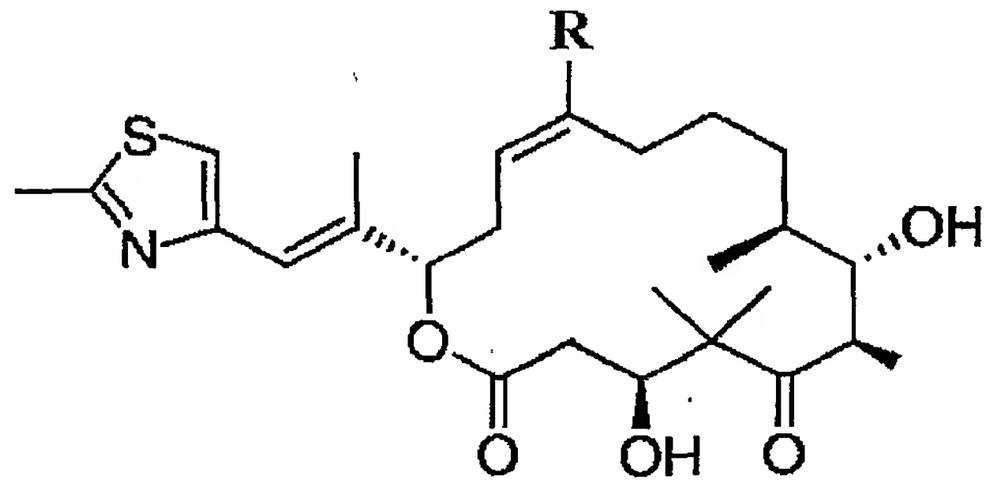
EPOTHILONE DERIVATIVES AND METHODS FOR MAKING AND USING THE SAME

BACKGROUND

Epothilone A (R = H) and Epothilone B (R = CH_3) are produced by *Sorangium cellulosum* strain So ce 90, the structures of which are shown below, and were the first of several epothilones to be isolated and characterized. Hofle *et al.*, 1996, *Angew. Chem. Int. Ed. Engl. 35*(13/14): 1567-1569.



Epothilone A and epothilone B possess many of the advantageous properties of taxol. As a result, there is significant interest in these and structurally related compounds as potential chemotherapeutic agents. The desoxy counterparts of epothilones A and B are known as epothilone C (R = H) and epothilone D (R = CH_3), and also exhibit similar anti-tumor activity but with less cytotoxicity. The structures of epothilones C and D are shown below.

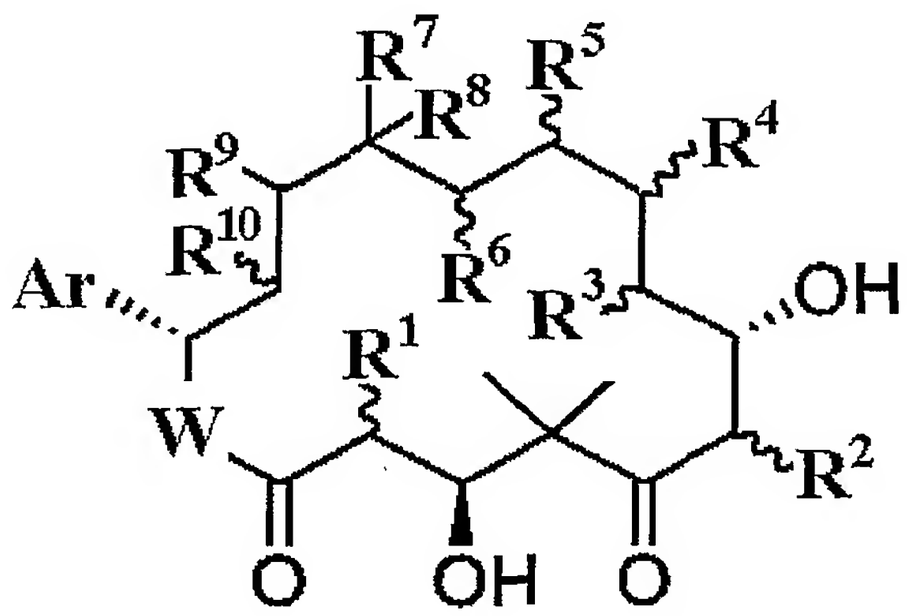


Although other naturally occurring epothilones have been described in the literature, these compounds are produced in exceedingly small amounts. For example, PCT publication WO 99/65913 describes 39 naturally occurring epothilones obtained from *Sorangium cellulosum* So ce 90 of which epothilones A, B, C, and D together account for approximately 98.9% of the total epothilones produced. The 35 other naturally occurring epothilone compounds together account for the remaining 1.1%.

Due to the increasing interest in epothilones as anti-cancer agents, novel derivatives of these compounds are needed and desired to more fully develop their therapeutic potential.

SUMMARY

The present invention relates to 16-membered macrocyclic compounds. In one aspect of the present invention, compounds of the formula



are provided wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl;

R^5 is hydrogen, oxo, or C_1-C_{10} aliphatic, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_{10} aliphatic, C_1-C_{10} alkylester, or halide;

R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid,

dioxolane and halogen, or optionally, R^6 and R^7 together form a 1, 3-dioxane that is optionally substituted at the 2-position;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

Ar is aryl; and,

W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl provided that at least one of R^4, R^5, and R^6 is not hydrogen. These compounds are cytotoxic agents and can be used to treat cancer and non-cancer disorders characterized by cellular hyperproliferation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to novel compounds that are useful for the treatment of cancer and other conditions characterized by abnormal cellular proliferation in a subject in need thereof. The present invention also relates to novel synthetic methods such as hydroxylation at the C-11 and C-26 positions and subsequent transformations thereof.

Definitions

Statements regarding the scope of the present invention and definitions of terms used herein are listed below. The definitions apply to the terms as they are used throughout this specification, unless otherwise limited in specific instances, either individually or as part of a larger group.

All stereoisomers of the inventive compounds are included within the scope of the invention, as pure compounds as well as mixtures thereof. Individual enantiomers, diastereomers, geometric isomers, and combinations and mixtures thereof are all encompassed by the present invention. Furthermore, some of the crystalline forms for the compounds may exist as polymorphs and as such are included in the present invention. In addition, some of the compounds may form solvates with water (i.e., hydrates) or common organic solvents, and such solvates are also encompassed within the scope of this invention.

Protected forms of the inventive compounds are included within the scope of the present invention. A variety of protecting groups are disclosed, for example, in T. H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, New York (1999), which is incorporated herein by reference in its entirety. For example, a hydroxy protected form of the inventive compounds are those where at least one of the hydroxyl groups is protected by a hydroxy protecting group. Illustrative hydroxyl protecting groups include but not limited to tetrahydropyranyl; benzyl; methylthiomethyl; ethylthiomethyl; pivaloyl; phenylsulfonyl; triphenylmethyl; trisubstituted silyl such as trimethyl silyl, triethylsilyl, tributylsilyl, tri-isopropylsilyl, t-butyldimethylsilyl, tri-t-butylsilyl, methyldiphenylsilyl, ethyldiphenylsilyl, t-butyldiphenylsilyl and the like; acyl and aroyl such as acetyl, pivaloylbenzoyl, 4-methoxybenzoyl, 4-nitrobenzoyl and aliphatic acylaryl and the like. Keto groups in the inventive compounds may similarly be protected.

The present invention includes within its scope prodrugs of the compounds of this invention. In general, such prodrugs are functional derivatives of the compounds that are readily convertible *in vivo* into the required compound. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various disorders described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to a subject in need thereof. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", H. Bundgaard ed., Elsevier, 1985.

As used herein, the term "aliphatic" refers to saturated and unsaturated straight chained, branched chain, cyclic, or polycyclic hydrocarbons that may be optionally substituted at one or more positions. Illustrative examples of aliphatic groups include alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. The term "alkyl" refers to straight or branched chain saturated hydrocarbon substituent. "Alkenyl" refers to a straight or branched chain hydrocarbon substituent with at least one carbon-carbon double bond. "Alkynyl" refers to a straight or branched chain hydrocarbon substituent with at least one carbon-carbon triple bound.

The term "aryl" refers to monocyclic or polycyclic groups having at least one aromatic ring structure that optionally include one ore more heteroatoms and preferably include three to fourteen carbon atoms. Aryl substituents may optionally be substituted at one or more positions. Illustrative examples of aryl groups include but are not limited to: furanyl, imidazolyl, indanyl, indenyl, indolyl, isooxazolyl, isoquinolinyl, naphthyl, oxazolyl, oxadiazolyl, phenyl, pyrazinyl, pyridyl, pyrimidinyl, pyrrolyl, pyrazolyl, quinolyl, quinoxalyl, tetrahydronaphththyl, tetrazolyl, thiazolyl, thienyl, and the like.

The aliphatic (i.e., alkyl, alkenyl, etc.) and aryl moieties may be optionally substituted with one or more substituents, preferably from one to five substituents, more preferably from one to three substituents, and most preferably from one to two substituents. The definition of any substituent or variable at a particular location in a molecule is independent of its definitions elsewhere in that molecule. It is understood that substituents and substitution patterns on the compounds of this invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth herein. Examples of suitable substituents include but are not limited to: alkyl, alkenyl, alkynyl, aryl, halo; trifluoromethyl; trifluoromethoxy; hydroxy; alkoxy; cycloalkoxy; heterocyclooxy; oxo; alkanoyl (-C(=O)-alkyl which is also referred to as "acyl")); aryloxy; alkanoyloxy; amino; alkylamino; arylamino; aralkylamino; cycloalkylamino; heterocycloamino; disubstituted amines in which the two amino substituents are selected from alkyl, aryl, or aralkyl; alkanoylamino; aroylamino; aralkanoylamino; substituted alkanoylamino; substituted arylamino; substituted aralkanoylamino; thiol; alkylthio; arylthio; aralkylthio; cycloalkylthio; heterocyclothio; alkylthiono; arylthiono; aralkylthiono; alkylsulfonyl; arylsulfonyl; aralkylsulfonyl; sulfonamido (e.g., SO_2NH_2); substituted sulfonamido; nitro; cyano; carboxy; carbamyl (e.g., $CONH_2$); substituted carbamyl (e.g., -C(=O)NRR' where R and R' are each independently hydrogen, alkyl, aryl, aralkyl and the like); alkoxycarbonyl, aryl, substituted aryl, guanidino, and heterocyclo such as indoyl, imidazolyl, furyl, thienyl, thiazolyl, pyrrolidyl, pyridyl, pyrimidyl and the like. Where applicable, the substituent may be further substituted such as with, alkyl, alkoxy, aryl, aralkyl, halogen, hydroxy and the like.

The terms "alkylaryl" or "arylalkyl" refer to an aryl group with an aliphatic substituent that is bonded to the compound through the aliphatic group. An illustrative example of an alkylaryl or arylalkyl group is benzyl, a phenyl with a methyl group that is bonded to the compound through the methyl group (—CH_2Ph where Ph is phenyl).

The term "acyl" refers to —C(=O)R where R is an aliphatic group, preferably a C_1-C_6 moiety.

The term "alkoxy" refers to —OR wherein O is oxygen and R is an aliphatic group.

The term "alkylester" refers to —OC(=O)R where R is an aliphatic group.

The term "aminoalkyl" refers to —RNH_2 where R is an aliphatic moiety.

The terms "halogen," "halo", or "halide" refer to fluorine, chlorine, bromine and iodine.

The term "haloalkyl" refers to —RX where R is an aliphatic moiety and X is one or more halogens.

The term "hydroxyalkyl" refers to —ROH where R is an aliphatic moiety.

The term "oxo" refers to a carbonyl oxygen (=O).

In addition to the explicit substitutions at the above-described groups, the inventive compounds may include other substitutions where applicable. For example, the lactone or lactam backbone or backbone substituents may be additionally substituted (*e.g.*, by replacing one of the hydrogens or by derivatizing a non-hydrogen group) with one or more substituents such as C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group. Illustrative examples of suitable functional groups include but are not limited to: acetal, alcohol, aldehyde, amide, amine, boronate, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, cyanohydrin, disulfide, enamine, ester, ether, halogen, hydrazide, hydrazone, imide, imido, imine, isocyanate, ketal, ketone, nitro, oxime, phosphine,

phosphonate, phosphonic acid, quaternary ammonium, sulfenyl, sulfide, sulfone, sulfonic acid, thiol, and the like.

The term "purified" as used herein to refer to a compound of the present invention, means that the compound is in a preparation in which the compound forms a major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more by weight of the components in the composition.

The term "subject" as used herein, refers to an animal, preferably a mammal, who has been the object of treatment, observation or experiment and most preferably a human who has been the object of treatment and/or observation.

The term "therapeutically effective amount" as used herein, means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

The term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product that results, directly or indirectly, from combinations of the specified ingredients in the specified amounts.

The term "pharmaceutically acceptable salt" is a salt of one or more of the inventive compounds. Suitable pharmaceutically acceptable salts of the compounds include acid addition salts which may, for example, be formed by mixing a solution of the compound with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts (e.g., sodium or potassium salts); alkaline earth metal salts (e.g., calcium or magnesium salts); and salts formed with suitable organic ligands (e.g., ammonium, quaternary ammonium and amine cations formed using counteranions such as

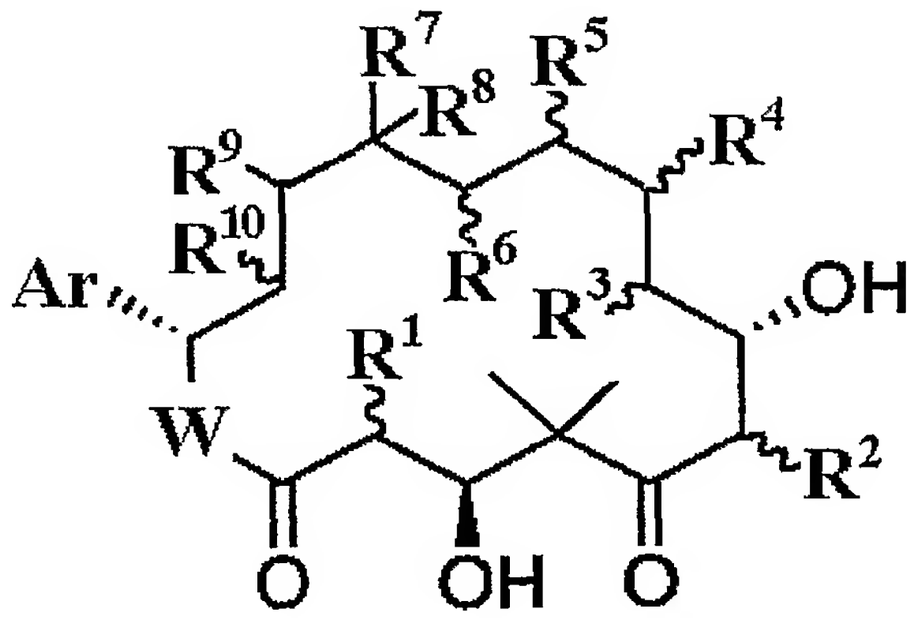
halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate). Illustrative examples of pharmaceutically acceptable salts include but are not limited to: acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like.

The term "pharmaceutically acceptable carrier" is a medium that is used to prepare a desired dosage form of the inventive compound. A pharmaceutically acceptable carrier includes solvents, diluents, or other liquid vehicle; dispersion or suspension aids; surface active agents; isotonic agents; thickening or emulsifying agents, preservatives; solid binders; lubricants and the like. Remington's Pharmaceutical Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton, Pa., 1975) and Handbook of Pharmaceutical Excipients, Third Edition, A.H. Kibbe, ed. (Amer. Pharmaceutical Assoc. 2000), both of which are incorporated herein by reference in their entireties, disclose various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof.

The term "pharmaceutically acceptable ester" is an ester that hydrolzyes *in vivo* into a compound of the present invention or a salt thereof. Illustrative examples of suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids such as formates, acetates, propionates, butyrates, acrylates, and ethylsuccinates.

Compounds of the Present Invention

The present invention provides compounds of the following formula



I

wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl;

R^5 is hydrogen, oxo, or C_1-C_{10} aliphatic, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_{10} aliphatic, C_1-C_{10} alkylester, or halide;

R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen, or optionally, R^6 and R^7 together form a 1,3-dioxane that is optionally substituted at the 2-position;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

Ar is aryl; and,

W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl.

In another embodiment, compounds of formula I are provided wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, Ar and W are as described previously provided that at least one of R^4, R^5 and R^6 is not hydrogen.

In another embodiment, compounds of formula I are provided wherein R^1, R^2, R^3, R^4, R^5, R^7, R^8, R^9, R^{10}, R^{11}, Ar and W are as described previously provided that R^6 is hydroxyl.

In another embodiment, compounds of formula I are provided wherein R^1, R^2, R^3, R^4, R^5, R^7, R^8, R^9, R^{10}, R^{11}, Ar and W are as described previously provided that R^6 is oxo.

In another embodiment, compounds of formula I are provided wherein R^1, R^2, R^3, R^4, R^5, R^7, R^8, R^9, R^{10}, R^{11}, Ar and W are as described previously provided that R^5 is oxo.

In another embodiment, compounds of formula I are provided wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_5 alkyl;

R^5 is hydrogen, oxo, or C_1-C_5 alkyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

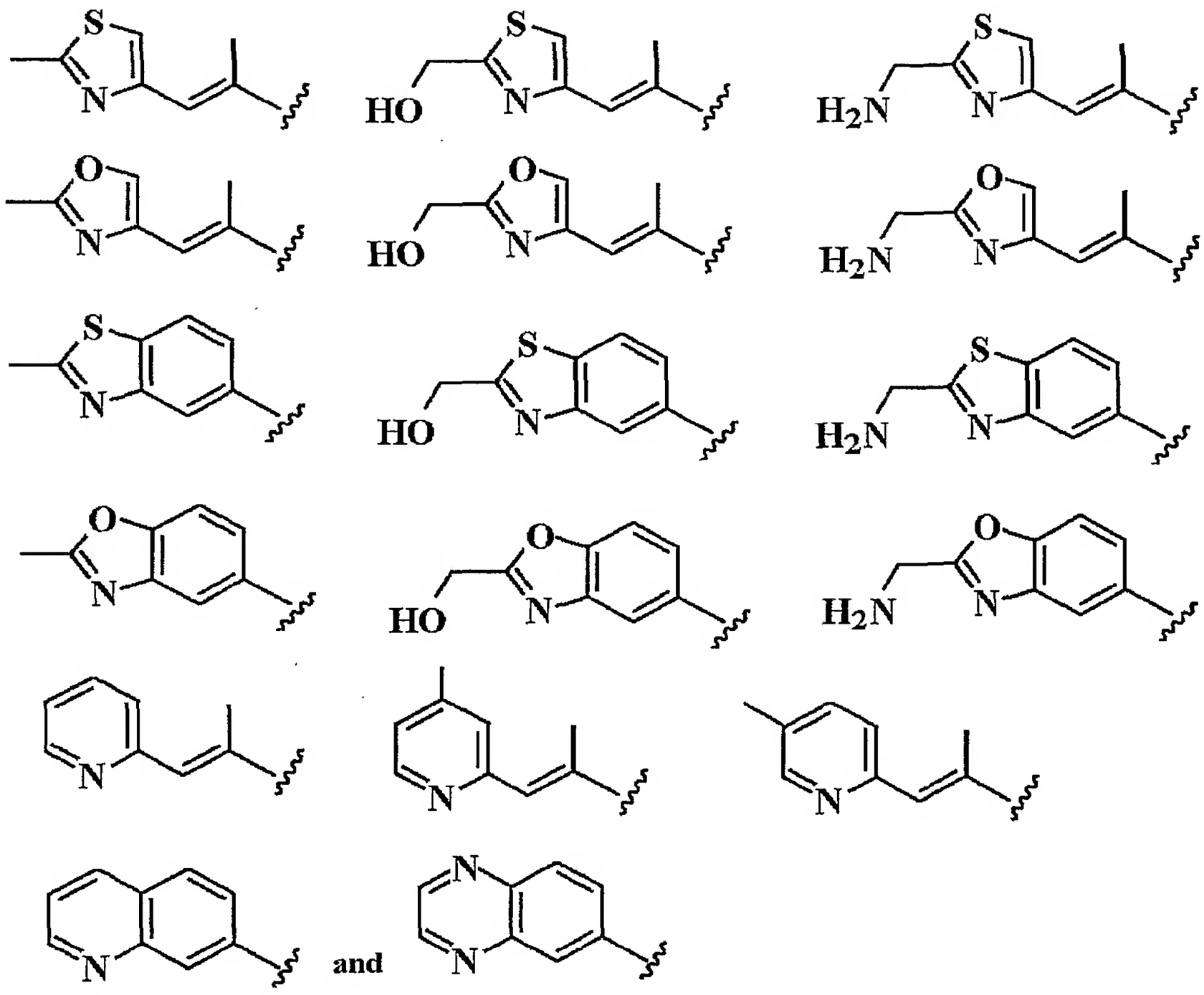
R^6 is hydrogen, hydroxyl, oxo, C_1-C_5 alkyl, or halide;

R^7 is hydrogen or C_1-C_5 alkyl that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen;

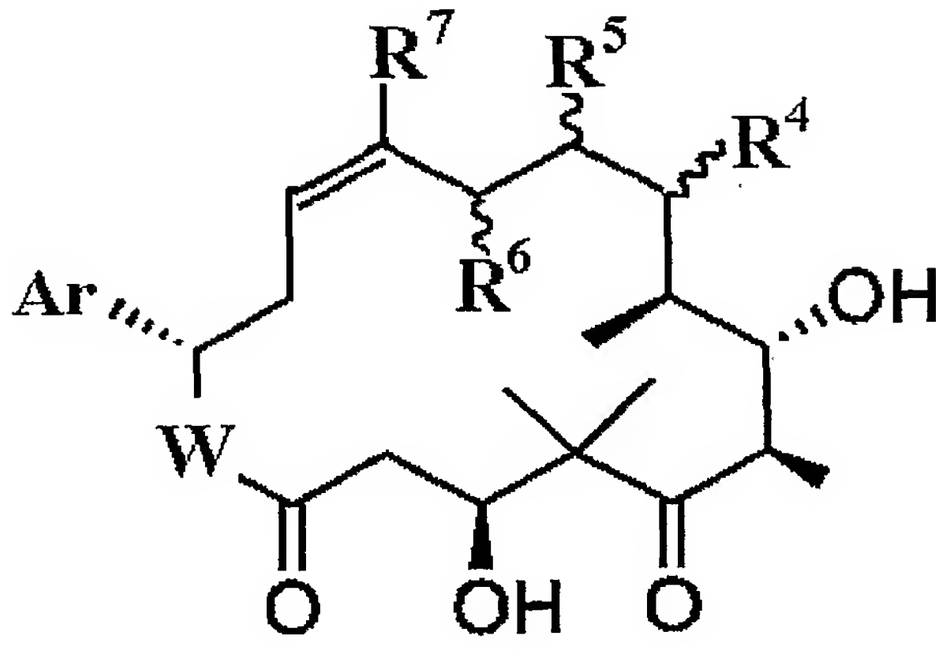
R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl; and,

Ar is selected from the group consisting of



In another aspect of the present invention, compounds of the formula



II

are provided wherein

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_5 alkyl;

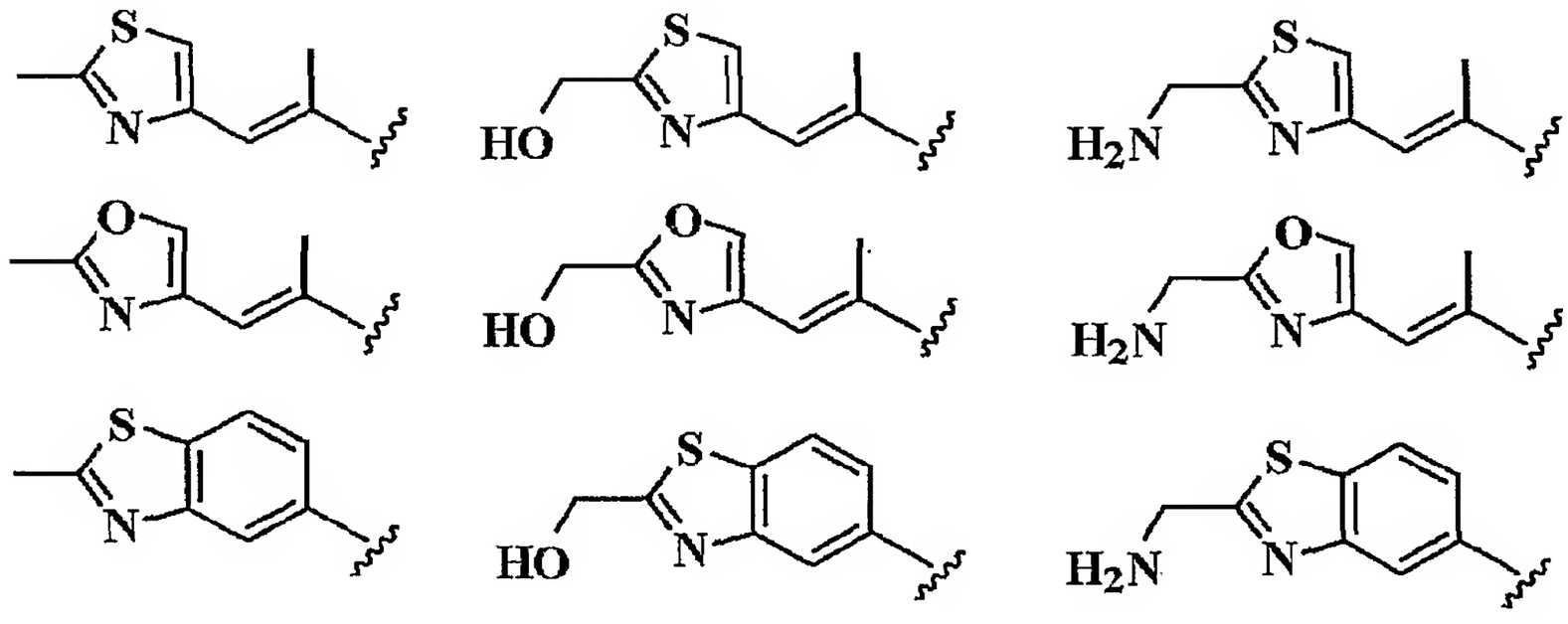
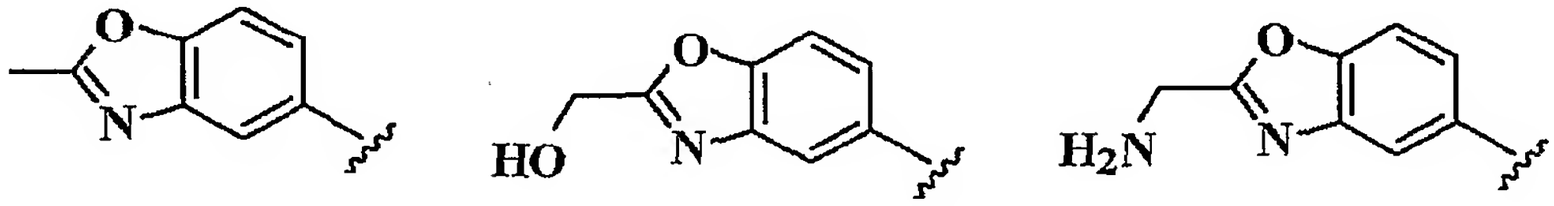
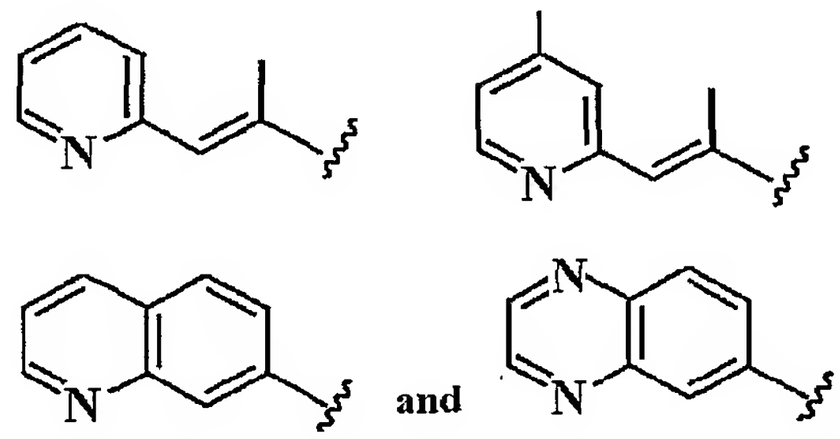
R^5 is hydrogen, oxo, or C_1-C_5 alkyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydoxyl, oxo, C_1-C_5 alkyl, or halide;

R^7 is hydrogen or C_1-C_5 alkyl that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen, or optionally, R^6 and R^7 together form a 1,3-dioxane that is optionally substituted at the 2-position;

W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl; and,

Ar is selected from the group consisting of




and

provided that R^5 or R^6 is not hydrogen.

In another embodiment, compound of formula II are provided wherein R^4, R^5, R^6, R^7, Ar and W are as described previously provided that R^5 or R^6 is hydroxyl or oxo.

In another embodiment, compound of formula II are provided wherein R^4, R^5, R^6, R^7, Ar and W are as described previously provided that R^6 is hydroxyl.

In another embodiment, compound of formula II are provided wherein R^4, R^5, R^6, R^7, Ar and W are as described previously provided that R^6 is oxo.

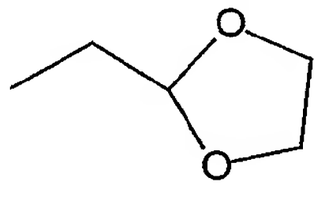
In another embodiment, compound of formula II are provided wherein R^4, R^5, R^6, R^7, Ar and W are as described previously provided that R^5 is oxo.

In another embodiment, compounds of formula II are provided wherein:

R^4 is hydrogen or NRR' where R and R' are independently hydrogen or methyl;

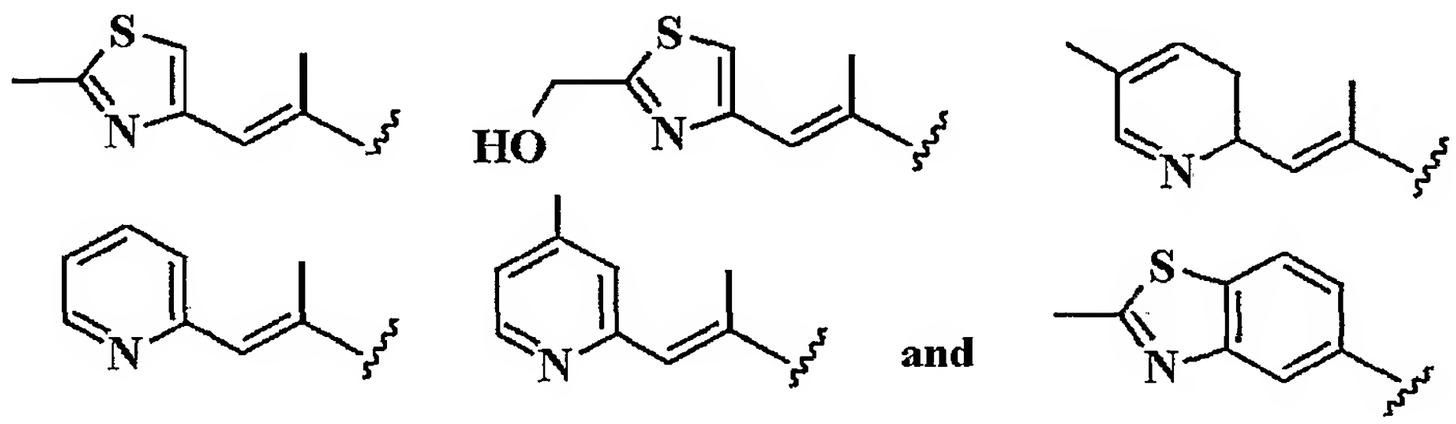
R^5 is hydrogen, oxo, or methyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, methyl, or fluoro;

R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, trifluoromethyl, $-CH_2CHO$, or  , or optionally, R^6 and R^7 together form a 1,3-dioxane;

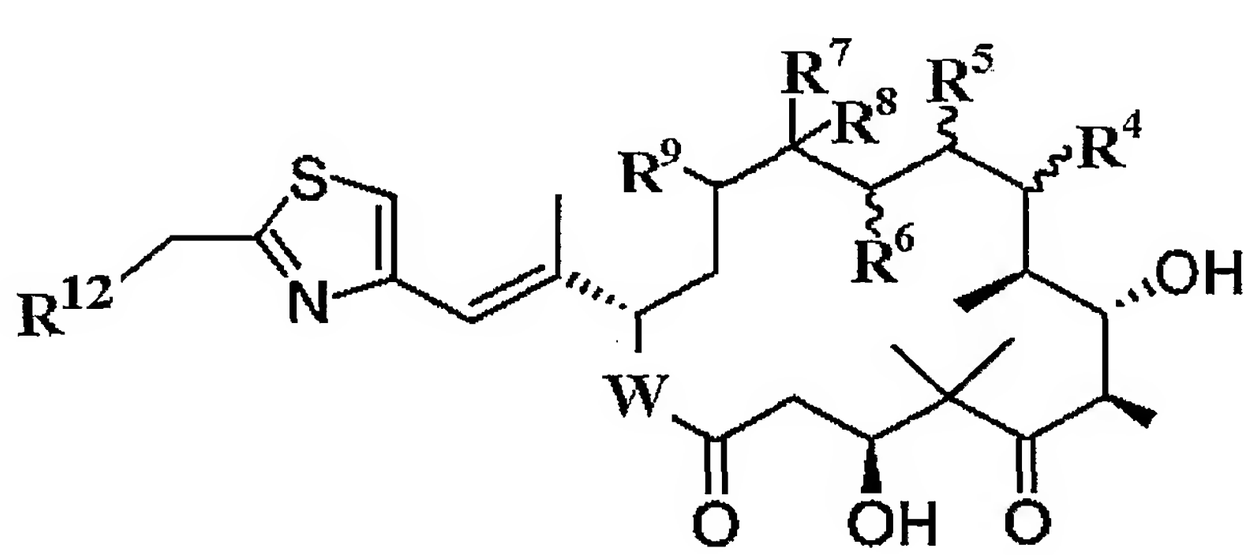
W is O or NH; and,

Ar is selected from the group consisting of



provided that R^5 or R^6 is not hydrogen..

In another aspect of the present invention, compounds of the formula



III

are provided wherein:

R^4 is hydrogen, oxo, or NRR' where R and R' are independently hydrogen or C_1-C_5 alkyl;

R^5 is hydrogen, oxo, C_1-C_5 alkyl, or optionally, R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_5 alkyl or halide;

R^7 is hydrogen or C_1-C_5 alkyl optionally substituted with alcohol, aldehyde, amine dioxalane, halide, or methoxy, or optionally, R^6 and R^7 together form a 1,3-dioxane that is optionally substituted at the 2-position;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

R^{12} is hydrogen, hydroxyl, or halide;

W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl,

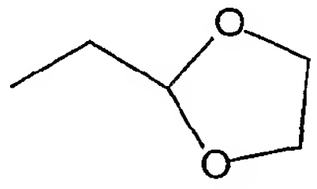
provided that R^5 or R^6 is not hydrogen.

In another embodiment, compounds of formula III are provided wherein

R^4 is hydrogen or NRR' where R and R' are independently hydrogen or methyl;

R^5 is hydrogen, oxo, or methyl, or optionally, R^4 and R^5 together form a carbon-carbon double bond;

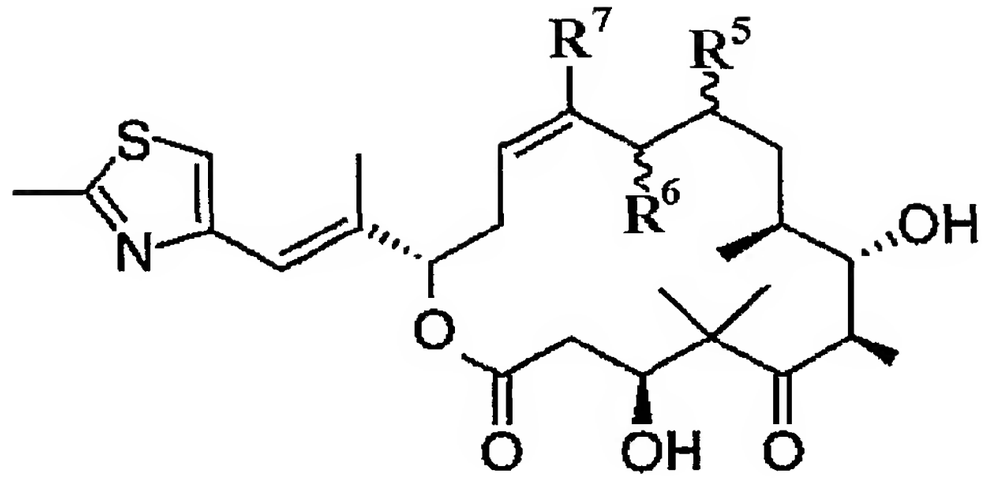
R^6 is hydrogen, hydroxyl, oxo, methyl or fluoro;

R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, trifluoromethyl, $-CH_2CHO$, or  , or optionally, R^6 and R^7 together form a 1,3-dioxane;

W is O or NH;

provided that R^5 or R^6 is not hydrogen..

In another aspect of the present invention, compounds of the formula



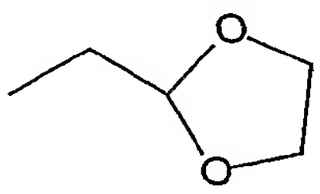
IV

are provided wherein

R^5 is hydrogen, oxo, or methyl;

R^6 is hydrogen, hydroxyl, oxo, fluoro or methyl; and,

R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, $-CH_2CHO$, or



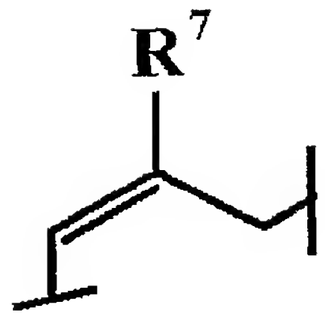
, or optionally, R^6 and R^7 together form a 1,3-dioxane that is optionally substituted at the 2-position,

provided that R^5 or R^6 is not hydrogen.

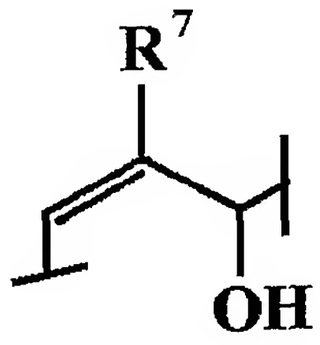
The compounds of the present invention are cytotoxic agents and may be used in any suitable manner including but not limited to as anti-cancer agents. An illustrative assay for assessing the degree of cytotoxicity and tubulin polymerization is described in Example 1.

Starting Materials

The synthetic methods of the present invention can be used with any epothilone compound having a double bond as part of its macrocycle to obtain a corresponding compound having a hydroxyl group at a carbon adjacent to said double bond. The newly added hydroxyl group then can be used as a chemical handle to obtain compounds having modifications at this and other adjacent positions. In one aspect of the present invention, epothilone compounds comprising the fragment

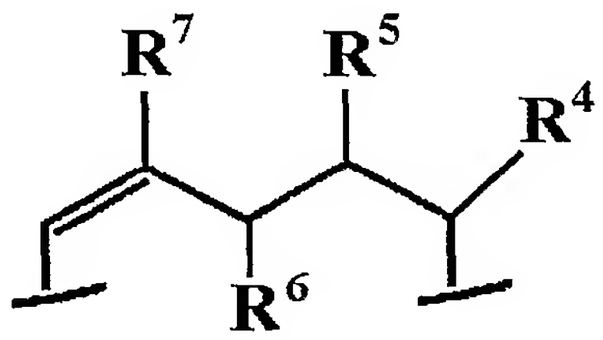


where R^7 is as previously defined, are used to obtain compounds comprising the



fragment

This compound is used to make further derivatives including those compounds comprising the fragment



where R^4, R^5, R^6 and R^7 are as previously defined.

The inventive methods can be used with epothilones C and D and with any other naturally occurring epothilone compounds having a double bond. For example, PCT Publication WO 99/65913 (which is incorporated herein by reference in its entirety) describes 39 naturally occurring epothilones obtained from *Sorangium cellulosum* So ce 90. Other suitable epothilones described by WO 99/65913 include: epothilone H_1, epothilone H_2, epothilone C_1, epothilone D_1, epothilone C_2, epothilone D_2, epothilone C_3, epothilone C_4, epothilone C_5, epothilone D_5, epothilone C_7, epothilone C_8, epothilone C_9, trans-epothilone C_1, trans-epothilone C_2, epothilone I_1, epothilone I_2, epothilone I_3, epothilone I_4, epothilone I_5, epothilone I_6, and epothilone K. Naturally occurring epothilones possessing an epoxide can be converted into their double bond counterparts using a deoxygenation method described by PCT Publication No. WO/99/43653 which is incorporated herein by reference. Briefly, the deoxygenation method comprises reacting the epoxy-containing

epothilones with a zinc/copper couple typically in the present of a polar solvent such as isopropanol and water.

Deposits of *Sorangium cellulosum* strain So ce90 from which epothilones were first extracted exist at the German Collection of Microorganisms as DSM 6773 (PCT publication WO 93/10121) and DSM 11999 (PCT publication WO 99/42602), a mutated version of DSM 6773 which allegedly displays increased production of epothilones A and B over the wild type strain. Fermentation conditions for *Sorangium* can be based on the protocols described in U.S. Patent No. 6,194,181, PCT Publication Nos. 93/10121, 97/19086, 98/22461, and 99/42602 and a publication by Gerth et al., 1996, *The Journal of Antibiotics*, 49: 560-563, each of which is incorporated herein by reference.

Epothilones and their derivatives can also be obtained from heterologous host cells using recombinant methods. Procedures for making epothilones in heterologous hosts such as *Myxococcus xanthus*, *Steptomyces lividians*, and *Pseudomonas fluorescens* are described in U.S. Serial No. 09/443,501 filed November 19, 1999 entitled RECOMBINANT METHODS AND MATERIALS FOR PRODUCING EPOTHILONE AND EPOTHILONE DERIVATIVES which is incorporated herein by reference. Among other things, the application provides the nucleotide sequence of the epothilone PKS and modification enzyme genes cloned from *Sorangium cellulosum* SMP44; cosmids containing overlapping fragments of the epothilone PKS and modification enzyme genes; plasmid pairs having the full complement of *epoA*, *epoB*, *epoC*, *epoD*, *epoE*, *epoF*, *epoK*, and *epoL* genes; and heterologous host cells for making epothilones and epothilone derivatives. Cosmids, pKOS35-70.1A2 (ATCC 203782), pKOS35-70.4 (ATCC 203781), pKOS35-70.8A3 (ATCC 203783), and pKOS35-79.85 (ATCC 203780); plasmid pair, pKOS039-124R (PTA-926) and pKOS039-126R (PTA-927); and strain K111-32.25 (PTA-1700) derived from *Myxococcus xanthus* containing all the epothilone genes and their promoters, have been deposited with the American Type Culture Collection ("ATCC"), Manassas, VA, USA. Additional procedures for making epothilones and epotholone derivatives in *Myxococcus xanthus* are described in U.S. Serial No. 09/560,367 filed April 28, 2000 entitled PRODUCTION OF POLYKETIDES, which is also incorporated herein by reference.

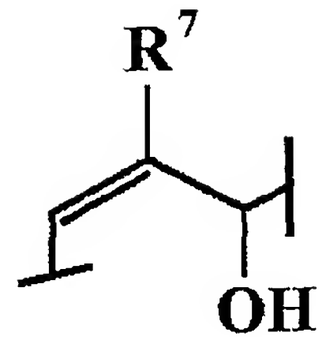
In addition, epothilone compounds can also be obtained from *de novo* chemical synthesis. The total synthesis of epothilones A and B have been achieved by several research groups including those of Danishefsky, Schinzer and Nicolaou. These syntheses are described for example by: U.S. Patent Nos. 6,156,905. 6,043,372 and 5,969,145 and PCT publications WO 98/08849, WO 98/25929, WO 99/01124 each of which is incorporated herein by reference.

Additional synthetic methods for making epothilone compounds are also described in PCT publications: 97/19086; 98/38192; 99/02514, 99/07692; 99/27890; 99/28324; 99/43653; 99/54318; 99/54319; 99/54330; 99/58534; 99/59985; 99/67252; 99/67253; 00/00485; 00/23452; 00/37473; 00/47584; 00/50423; 00/57874; 00/58254; 00/66589; 00/71521; 01/07439; and 01/27308, each of which is incorporated herein by reference.

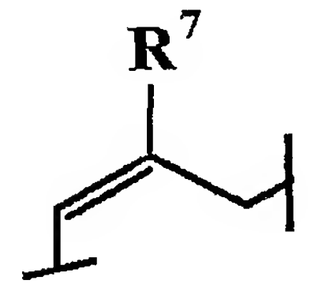
Synthetic Methods

General principles of organic chemistry including functional moieties and reactivity and common protocols are described by for example in Advanced Organic Chemistry 3rd Ed. by Jerry March (1985) which is incorporated herein by reference in its entirety. In addition, it will be appreciated by one of ordinary skill in the art that the synthetic methods described herein may use a variety of protecting groups whether or not they are explicitly described. A "protecting group" as used herein means a moiety used to block functional moiety such as oxygen, sulfur, or nitrogen so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. General principles including specific functional groups and their uses are described for example in T. H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons, New York (1999).

In one aspect of the present invention, a hydroxylation method is provided for making a compound comprising the fragment



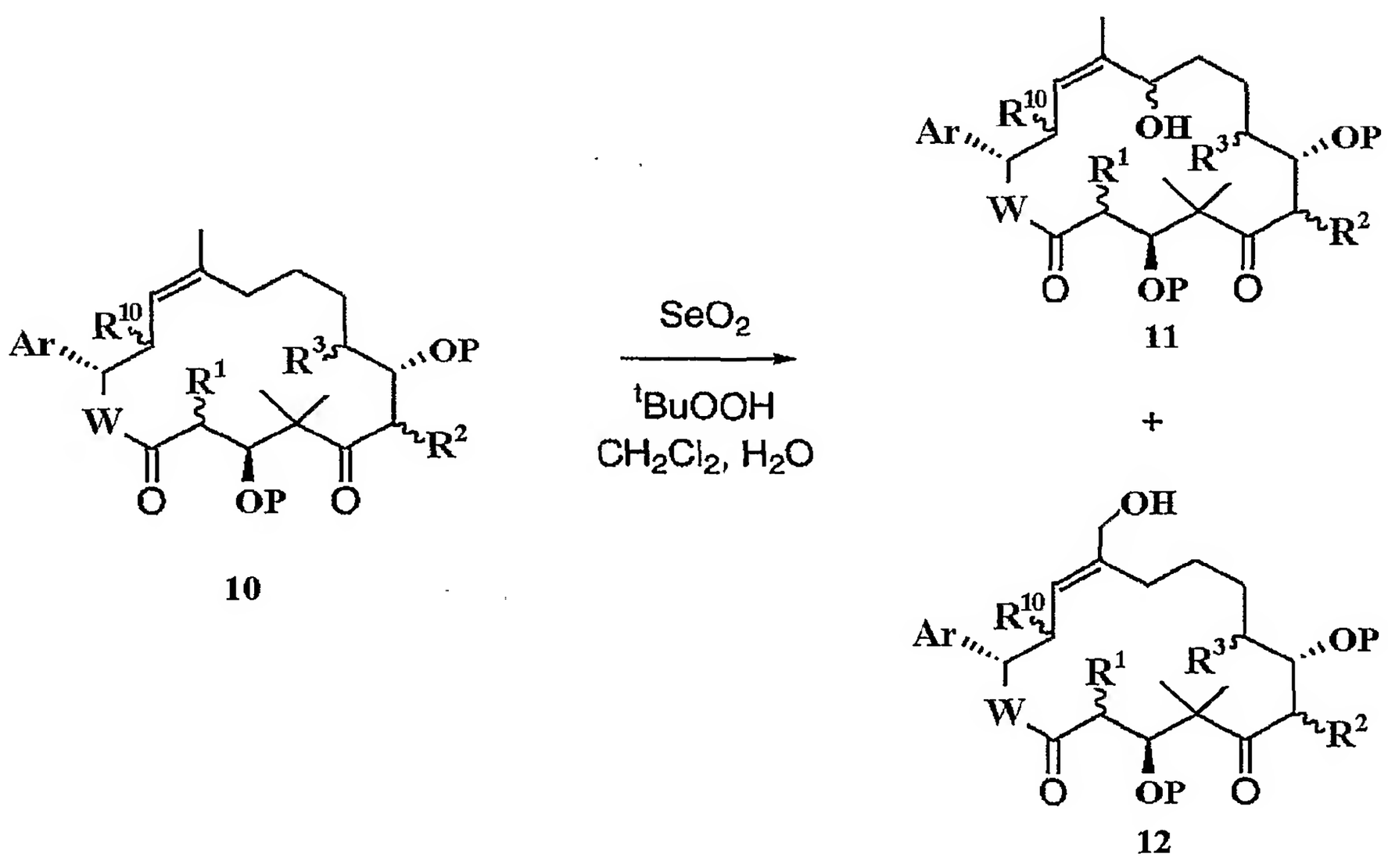
where R^7 is as previously defined, by treating a compound comprising the fragment



with a selective hydroxylating agent such as selenium dioxide.

Scheme 1 illustrates one embodiment of this method.

SCHEME 1



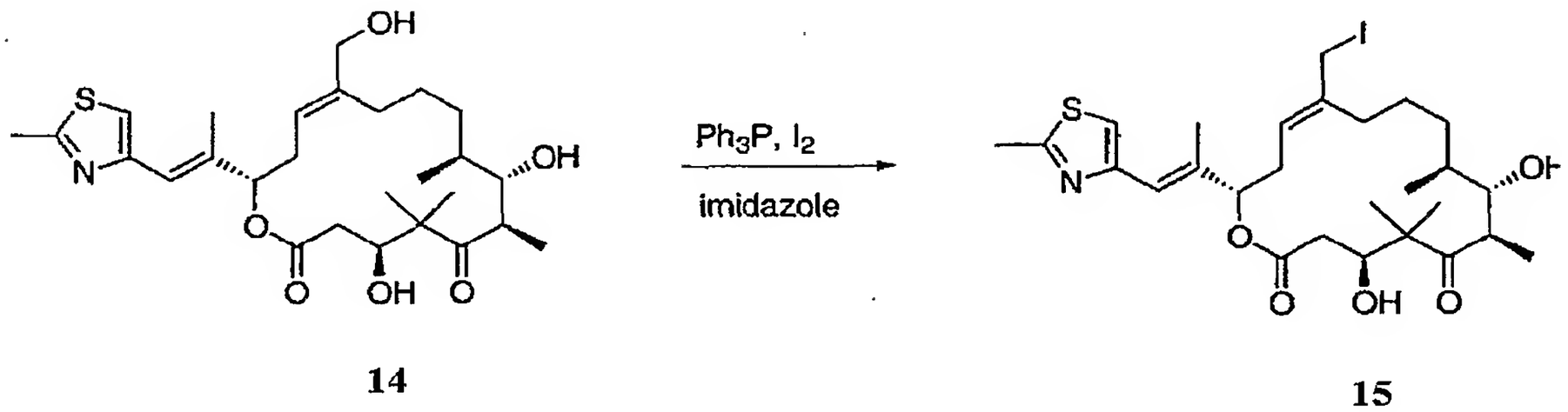
As shown in Scheme 1, treatment of desoxyepothilone **10** (where R^1, R^2, R^3, R^{10}, Ar, and W are as previously described and P is either hydrogen or a hydroxy protecting

group) with selenium dioxide under the described conditions results in two main products: a C-11 hydroxy compound **11** and a C-12 hydroxymethyl (also referred to as C-26 hydroxy) compound **12**. Under certain reaction conditions (particularly longer reaction times), a 11,26-bis(hydroxy) derivative of compound **10** is also formed (compound **13**, not shown in Scheme 1 but see Scheme 3B). The newly added hydroxyl groups of these products can be used as chemical handles for subsequent chemical transformations. Notably, this hydroxylation using selenium dioxide is very selective for the C-11 and C-26 positions. For example, when this method is applied to epothilone D, the hydroxylation productions are 11-hydroxy-epothilone D and 26-hydroxy-epothilone D. A 27-hydroxy-epothilone D product is not observed at any appreciable level.

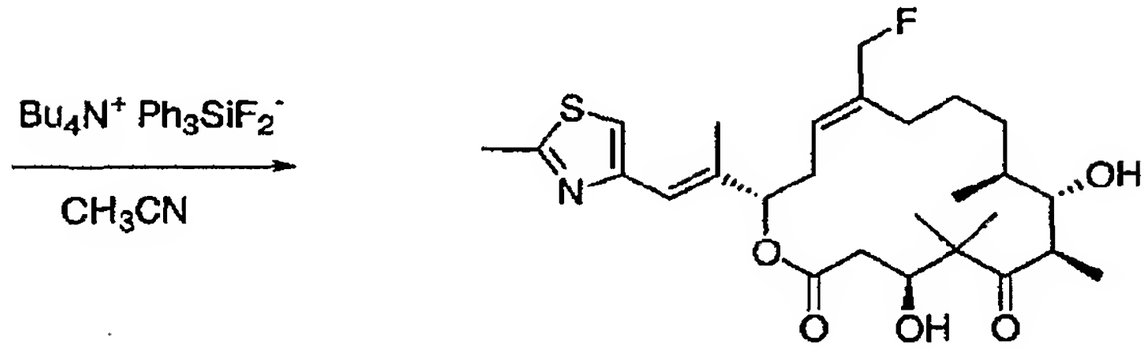
In another aspect of the present invention, methods are provided for converting C-12 hydroxymethyl containing compounds into C-12 halomethyl compounds. In one embodiment, a 12-hydroxymethyl (aka 26-hydroxy) compound **12** is converted into the corresponding 12-iodomethyl (aka 26-iodide) compound using imidazole, triphenylphosphine and iodine. The 12-iodomethyl (aka 26-iodide) compound can be converted into the 12-fluoromethyl (aka 26-fluoro) derivative using a source of nucleophilic fluoride such as tetrabutylammonium triphenyldifluorosilicate or $Bu_4N^+F^-$..

Scheme 2 illustrates this embodiment using a 12-hydroxymethyl (aka 26-hydroxy)-epothilone D **14** as an example.

SCHEME 2



14 15



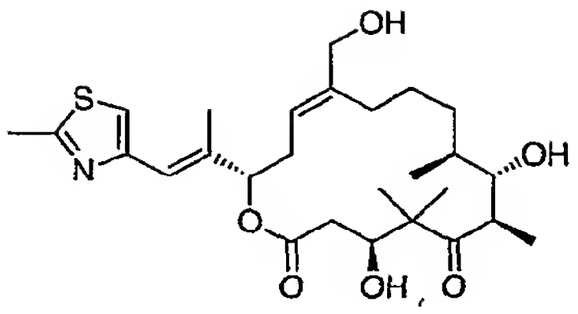
16

Compound **14** is treated with imidazole, triphenylphosphine and iodine to yield compound **15**. The iodinated compound is then treated with tetrabutylammonium triphenyldifluorosilicate to yield the C-12-fluoromethyl compound **16**.

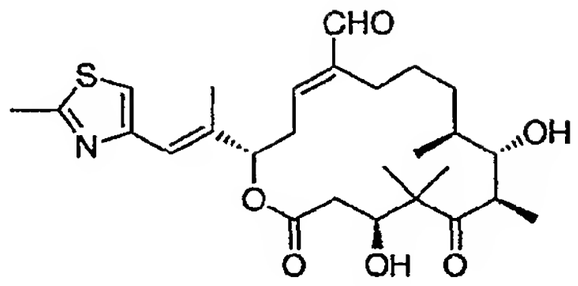
In another embodiment, the method outlined in Scheme 2 is used on 21, 26-dihydroxy-epothilone D (21-hydroxy version of compound 14) to yield 21, 26-diiodoepothilone D and 21, 26-difluoroepothilone D.

In another aspect of the present invention, methods are provided for converting C-12 hydroxymethyl containing compounds into C-12–(2-dioxolanylmethyl) containing compounds. Scheme 3A illustrates one embodiment using 12-hydroxymethyl- (aka 26-hydroxy) epothilone D as an example.

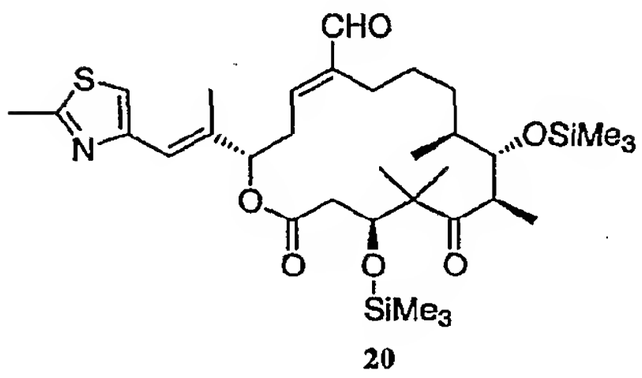
SCHEME 3A



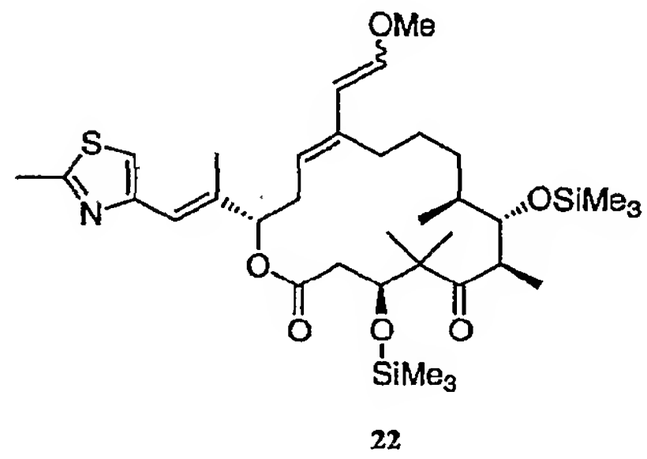
14



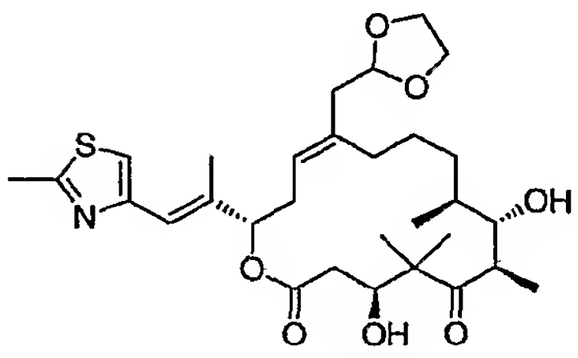
18



20



22

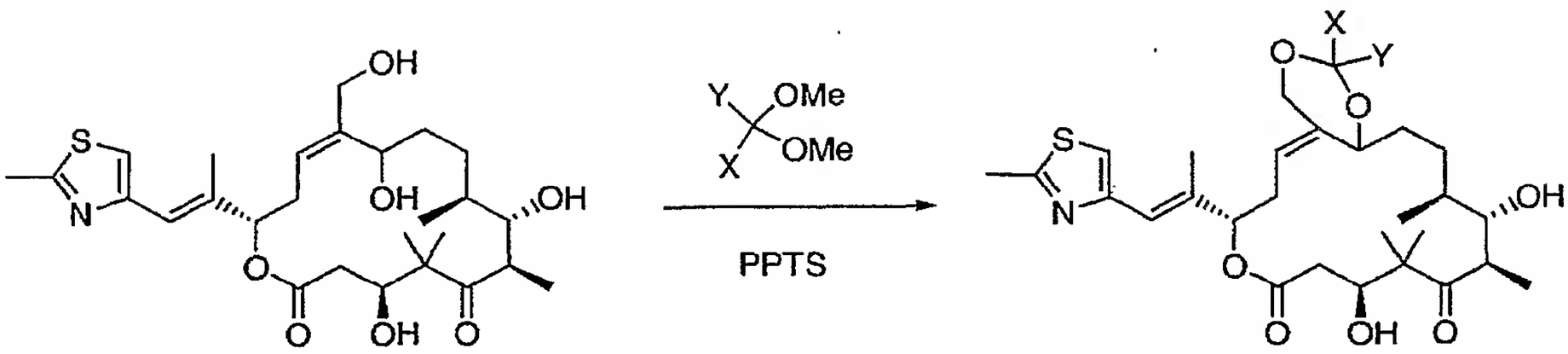


24

As shown in Scheme 3A, 26-hydroxy epothilone D **14** is oxidize to the corresponding 26-oxo epothilone D **18**. The C-3 and C-7 hydroxyl groups are protected (to form compound **20**) and then reacted with (methoxymethylidene)triphenylphosphorane chloride to yield compound **22**. Finally, the 3,7-bis(O-trimethylsilyl)-26-methoxymethylidene-epothilone **22** is reacted with ethylene glycol with acid catalysis to yield 26-(2-dioxolanyl)-epothilone D **24**.

In another aspect of the present invention, methods are provided for converting 11, 26-bishydroxy compounds into 1,3-dioxane-containing derivatives. Scheme 3B illustrates one embodiment using 11, 26-bishydroxy-epothilone D as an example.

SCHEME 3B

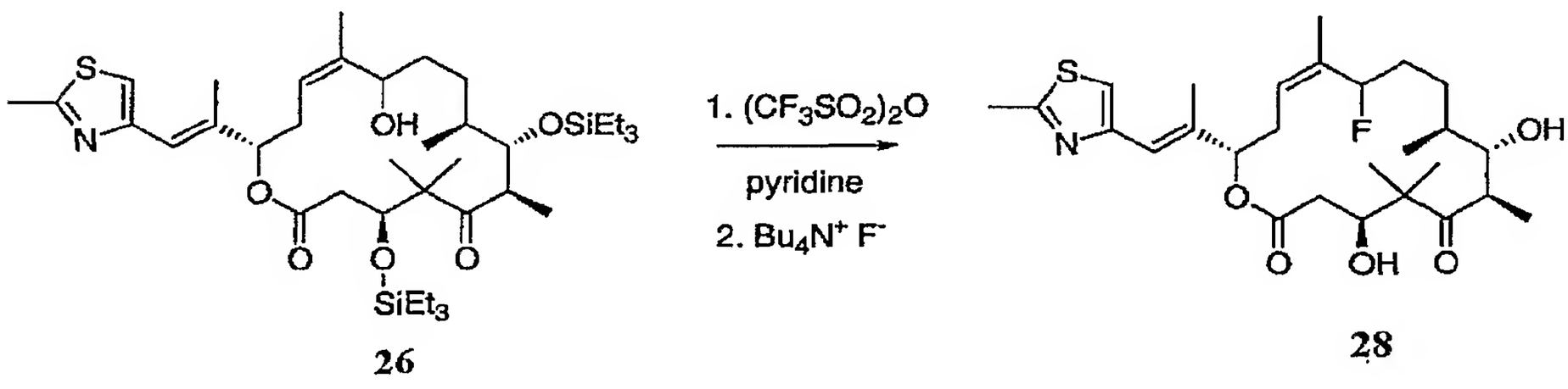


13 **17**

As shown in Scheme 3B, 11, 26-bishydroxy epothilone D **13** is treated with pyridinium p-toluenesulfonate ("PPTS") and an optionally substituted dimethoxymethane (where X and Y each independently is hydrogen, C_1-C_5 alkyl, or C_1-C_5 alkoxy) to yield a 1,3-dioxane derivative **17**.

In another aspect of the present invention, methods are provided for converting 11-hydroxy compounds into 11-halo compounds by treating a suitably protected form of compound **11** with an activating agent followed by a halogenating agent. Scheme 4 illustrates one embodiment using 11-hydroxy-epothilone D as an example.

SCHEME 4

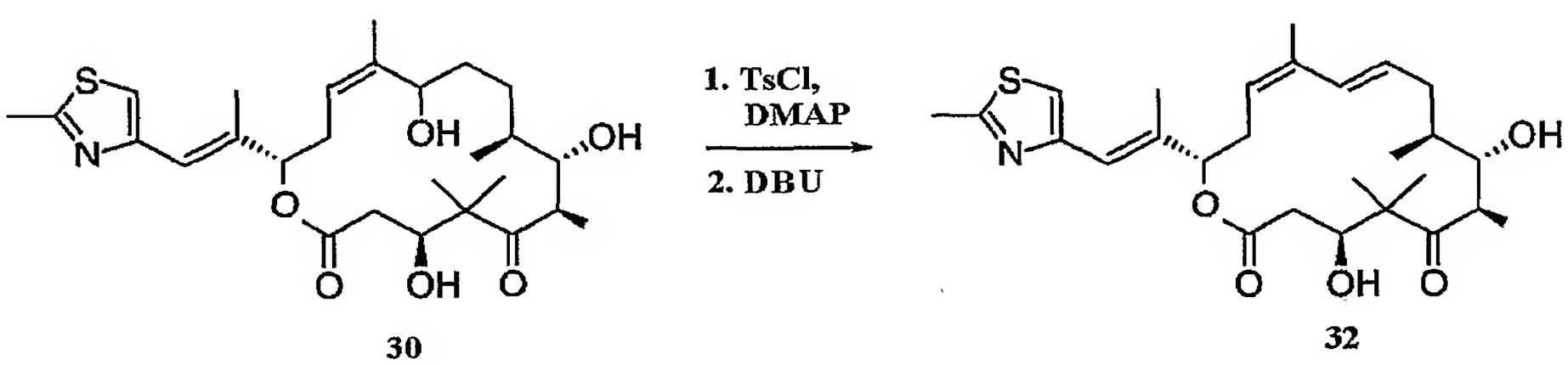


26 **28**

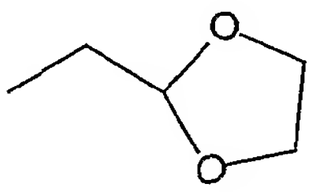
11-hydroxy-epothilone D is protected using for example, chlorotriethylsilane and 4-dimethylaminopyridine to yield compound **26**. The 3,7-bis(O-triethylsilyl)-11-hydroxyepothilone D **26** is treated with pyridine and trifluoromethanesulfonic anhydride and then with tetrabutylammonium fluoride to yield 11-fluoro-epothilone D **28**.

In another aspect of the present invention, methods are provided for converting 11-hydroxy compounds into 10, 11-anhydrocompounds. Scheme 5 illustrates one embodiment of this method using 11-hydroxy-epothilone D as an example.

SCHEME 5

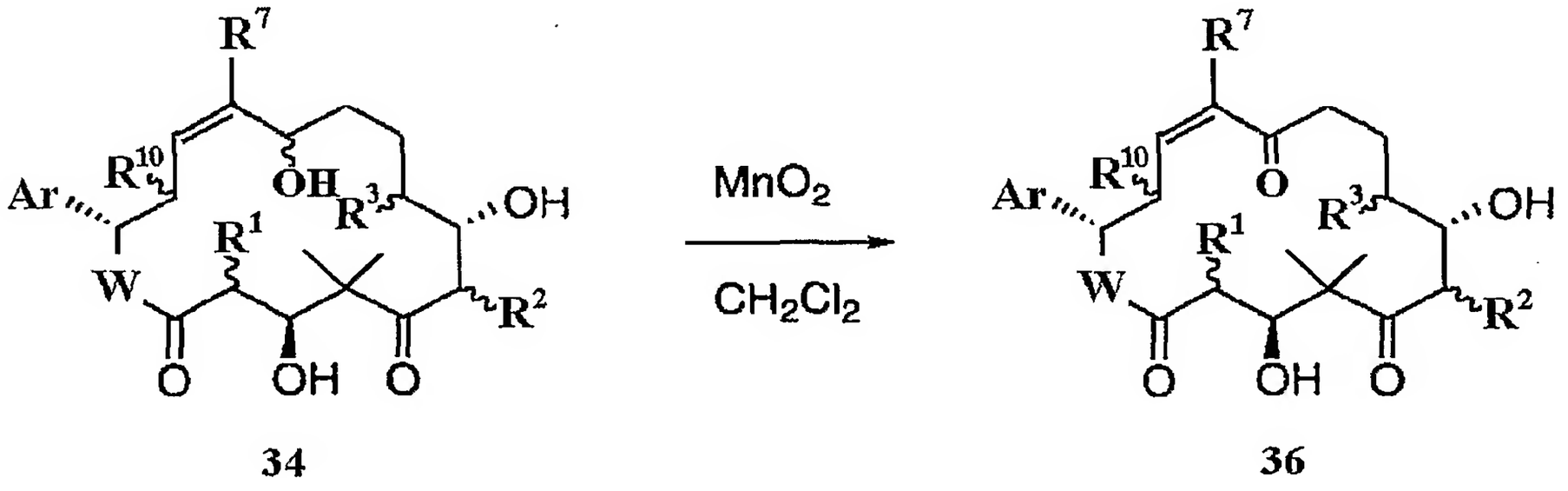


30 **32**

As shown by Scheme 5, 11-hydroxy-epothilone D **30** is treated with toluenesulfonyl chloride and 4-(dimethylamino)pyridine ("DMAP") and then with 1,8-diazabicyclo[5.4.0]undec-7-ene ("DBU") to yield 10,11-dehydro-epothilone D **32**. Compound **32** can also be obtained *M. xanthus* strains that make epothilones. For example, K111-72-4.4 expresses the epothilone polyketide synthase and contains an *epoK* gene with an inactivating in frame deletion. Strain K111-72-4.4 (PTA-2713) was deposited in the ATCC on November 21, 2000. See also U.S. Serial No. 09/___,___ filed on April 3, 2001 entitled EPOTHILONE COMPOUNDS AND METHODS OF MAKING AND USING THE SAME by inventors Robert Arslanian, John Carney and Brian Metcalf which is incorporated herein by reference (Morrison & Foerster Attorney Docket No. 20066.00). In another embodiment, this method is applied to compounds of formula II wherein R^4 and R^5 are each hydrogen; R^6 is hydroxyl; and, R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, $-CH_2CHO$, or  and W is O, to yield the corresponding 10, 11-anhydro product.

In another aspect of the present invention, methods are provided for converting 11-hydroxy compounds into 11-oxo compounds. Scheme 6 illustrates one embodiment of this method.

SCHEME 6

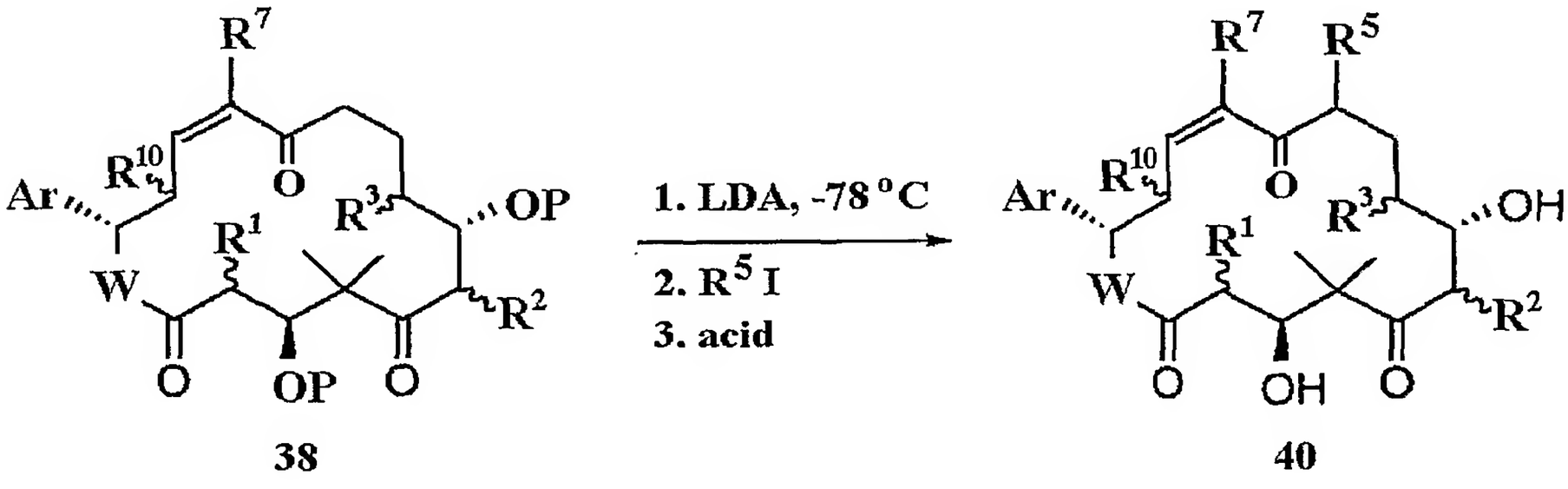


34 **36**

As shown in Scheme 6, the C-11 hydroxyl of compound **34** is oxidized using an oxidizing agent such as manganese dioxide to yield the corresponding 11-oxo compound **36**. The 11-oxo compound **36** in turn can be used to make other compounds of the present invention.

In another aspect of the present invention, methods are provided for alkylating (or arylating) 11-oxo compounds at C-10. Scheme 7 illustrates one embodiment of this method.

SCHEME 7

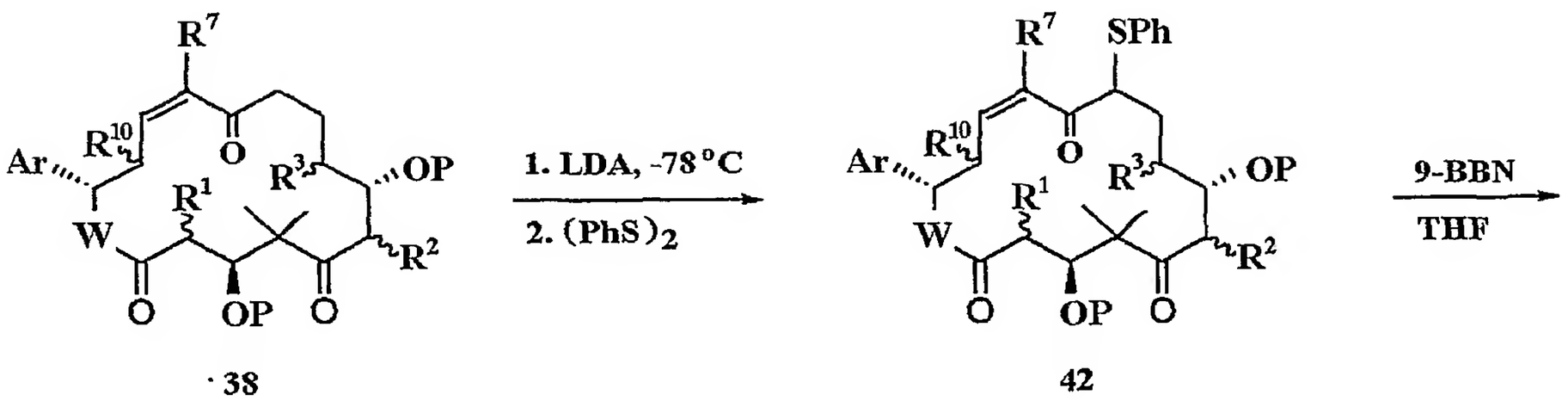


38 **40**

11-oxo compound **36** is protected using for example, chlorotriethylsilane and 4-dimethylaminopyridine to yield compound **38** where P is a hydroxy protecting group. The protected compound **38** is treated with lithium diisopropylamide ("LDA") and then treated with R^5I (where R^5 is an aliphatic or aryl moiety) to yield a compound with R^5 at the C-10 position. Deprotection using acid, for example a mixture of trifluoroacetic acid and CH_2Cl_2 or HF and CH_3CN, yields compound **40**.

In another aspect of the present invention, methods are provided for converting 11-oxo compounds into 10-oxo compounds using carbonyl transposition. Scheme 8 illustrates one embodiment of this method.

SCHEME 8



38 42

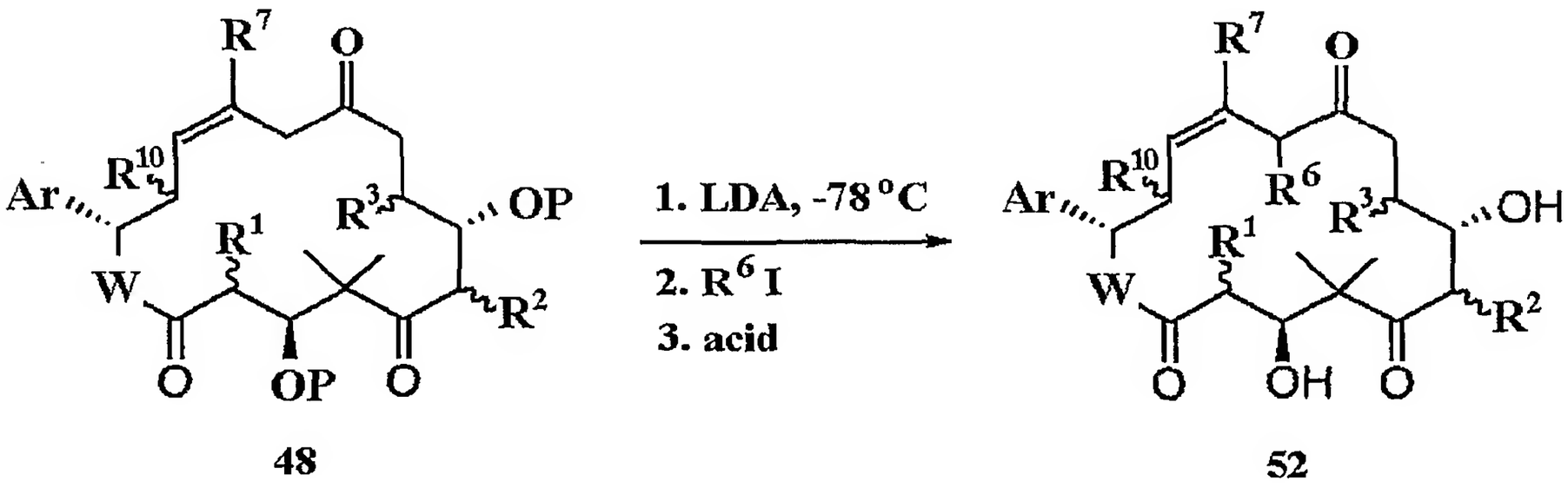
44 46

48 50

As shown in Scheme 8, protected 11-oxo compound **38** is treated with LDA and then treated with phenyldisulfide. The resulting 10-phenylsulfenyl compound **42** is treated with 9-borabicyclo[3.3.1]nonane ("9-BBN") in tetrahydrofuran ("THF") to yield 11-hydroxy compound **44**. The 11-hydroxy compound is treated with methanesulfonic anhydride and then with DBU to yield the 10, 11-dehydro compound **46**. Treatment of the resulting product with mercuric chloride and acetonitrile yields the protected 10-oxo compound which can be deprotected with acid such as trifluoroacetic acid to yield compound **50**.

In another aspect of the present invention, methods are provided for alkylating (or arylating) 10-oxo compounds at C-11. Scheme 9 illustrates one embodiment of this method.

SCHEME 9

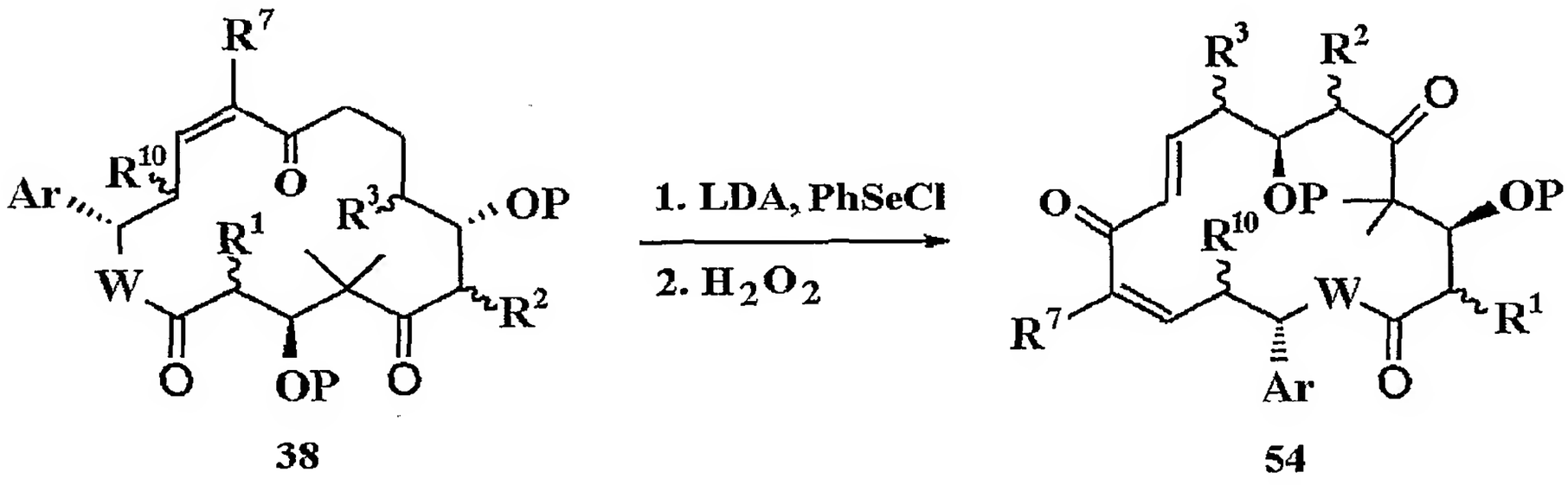


48 **52**

Protected 10-oxo compound **48** is treated with LDA and then treated with R^6I (where R^6 is an aliphatic or aryl moiety) to yield a compound with R^6 at the C-11 position. Deprotection using acid, for example a mixture of trifluoroacetic acid and CH_2Cl_2 or HF and CH_3CN, yields compound **52**.

In another aspect of the present invention, methods are provided for making 9,10-dehydro-11-oxo compounds. Scheme 10 illustrates one embodiment of this method.

SCHEME 10

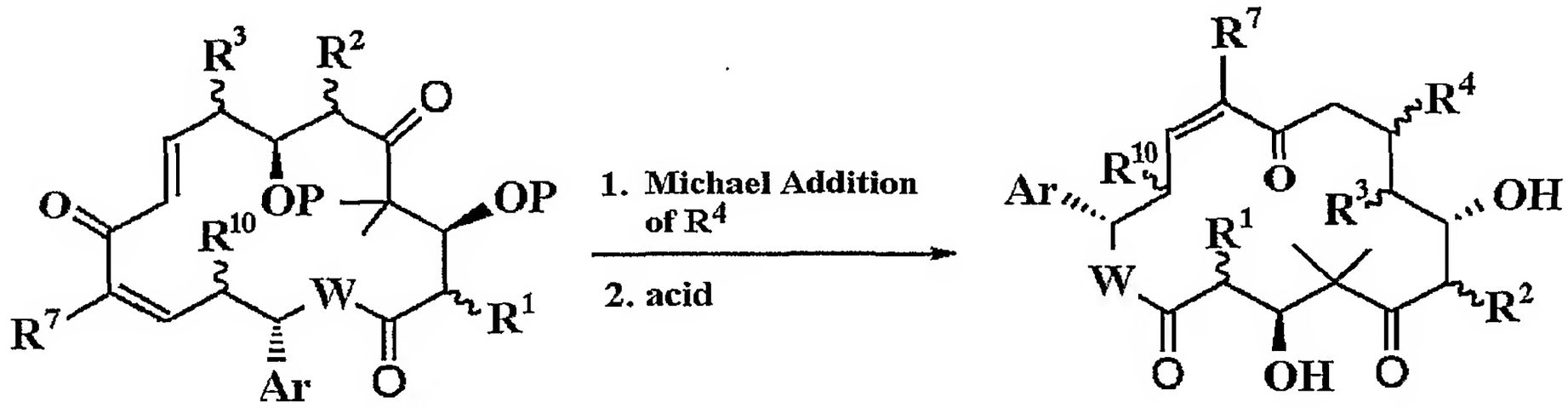


38 **54**

As shown in Scheme 10, the protected 11-oxo compound **38** is subject to a dehydrogenation reaction via selenoxide elimination to yield compound **54**. Deprotection using acid such as trifluoroacetic acid results in the 9, 10-dehydro-11-oxo compound.

In another aspect of the present invention, methods are provided for modifying 11-oxo compounds by adding a nucleophile at the C-9 position. Scheme 11 illustrates one embodiment of this method where a nucleophilic moiety is added using Michael addition.

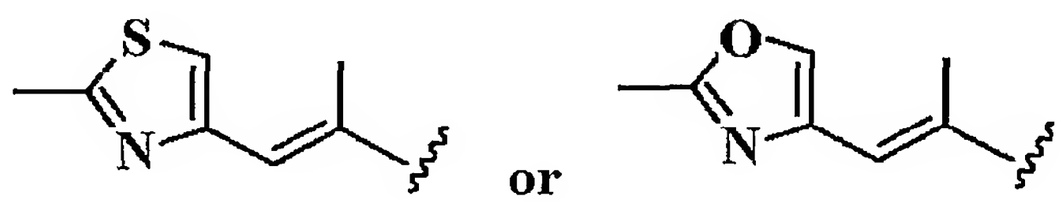
SCHEME 11



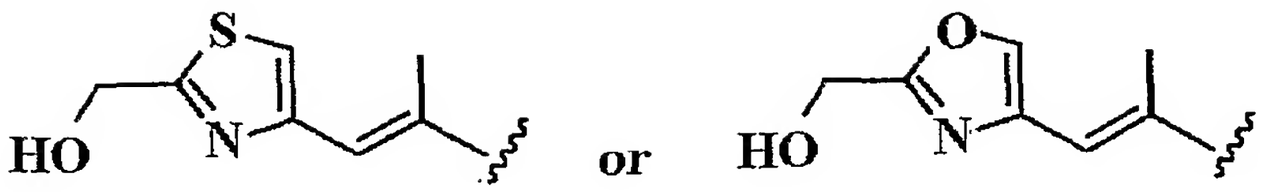
54 **56**

In one embodiment, the R^4 is a strong nucleophile. In another embodiment, R^4 is of the formula NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl. In another embodiment, R^4 is of the formula NRR' where R and R' are independently hydrogen or C_1-C_5 alkyl. In yet another embodiment, R^4 is of the formula NRR' where R and R' are independently hydrogen or methyl.

In another aspect of the present invention, compounds of formula I where Ar is



are hydroxylated using a microbially-derived hydroxylase to make the corresponding C-21 hydroxy compounds where Ar is

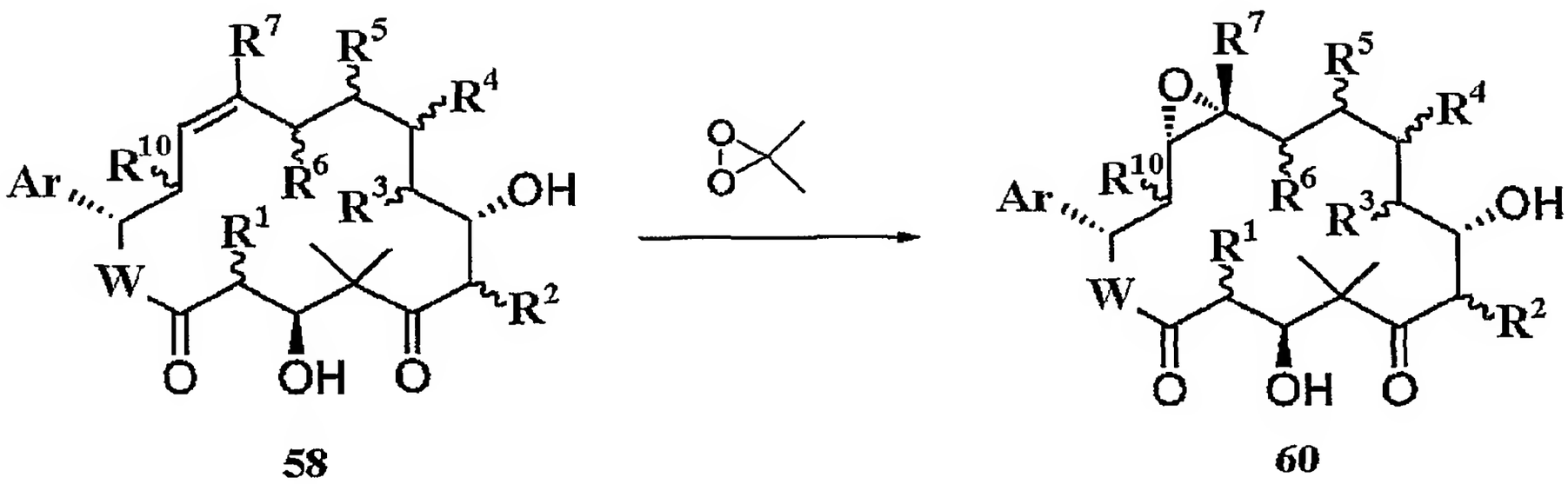


Protocols for effectuating such a transformation are described for example by PCT Publication No. WO 00/39276 which is incorporated herein in its entirety by reference, and by Example 24 herein.

In another embodiment, compounds of the invention having a carbon-carbon double bond at the positions corresponding to C-12 and C-13 of epothilones A-D can be epoxidated using EpoK or another P450 epoxidase. A general method for using EpoK for epoxidation is described by Example 5 of PCT publication WO 00/31247 which is incorporated herein by reference, and by Example 25 herein. Alternatively, the epoxidation reaction can occur by contacting an epothilone compound containing a double bond at a position that corresponds to the bond between carbon-12 and carbon 13 to a culture of cells that expresses a functional Epo K. Such cells include the myxobacterium *Sorangium cellulosum*. In particularly preferred embodiments, the *Sorangium cellulosum* expresses Epo K but does not contain a functional epothilone polyketide synthase ("PKS") gene. Such strains may be made by mutagenesis where one or more mutations in the epothilone PKS gene render it inoperative. Such mutants can occur naturally (which may be found by screening) or can be directed using either mutagens such as chemicals or irradation or by genetic manipulation. A particularly effective strategy for making strains with an inoperative epothilone PKS is homologous recombination as described by PCT publication WO 00/31247.

In another embodiment, the epoxidation reaction can occur using synthetic methods. For example, as shown by Scheme 12, desoxy compounds of the invention **58** can be transformed to the epoxy counterparts **60** by reacting the desoxy compounds with dimethyldioxirane.

SCHEME 12

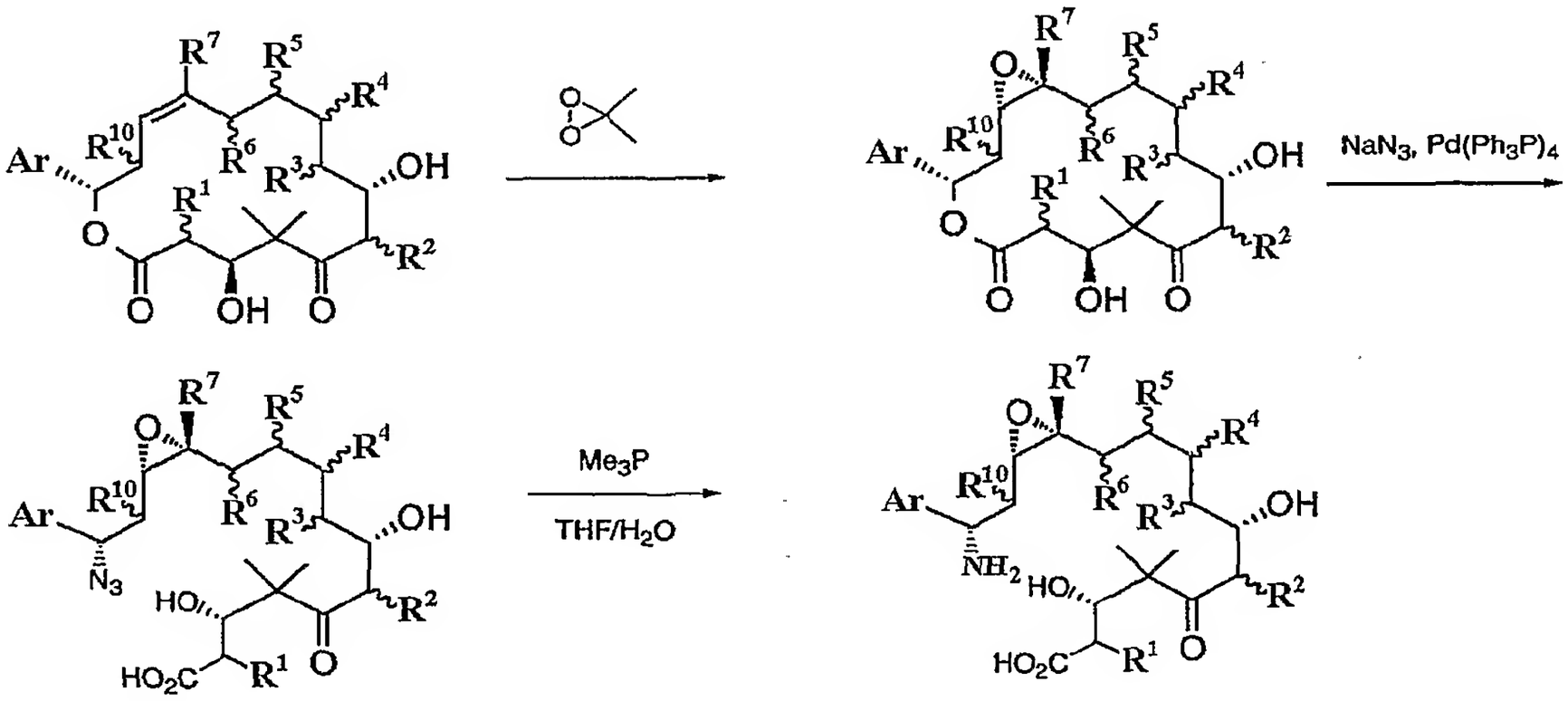


58 60

Example 26 describes this synthetic method in greater detail.

In another embodiment, macrolactams can be converted into the corresponding macrolactams for use as starting material in the practice of the present invention. In another embodiment, inventive macrolactams can be converted into the corresponding macrolactams which are also compounds of the present invention. As illustrated by Scheme 13, a desoxy macrolactone of the invention is epoxidized using dimethyldioxirane as previously described by Scheme 12 to provide the oxycounterpart.

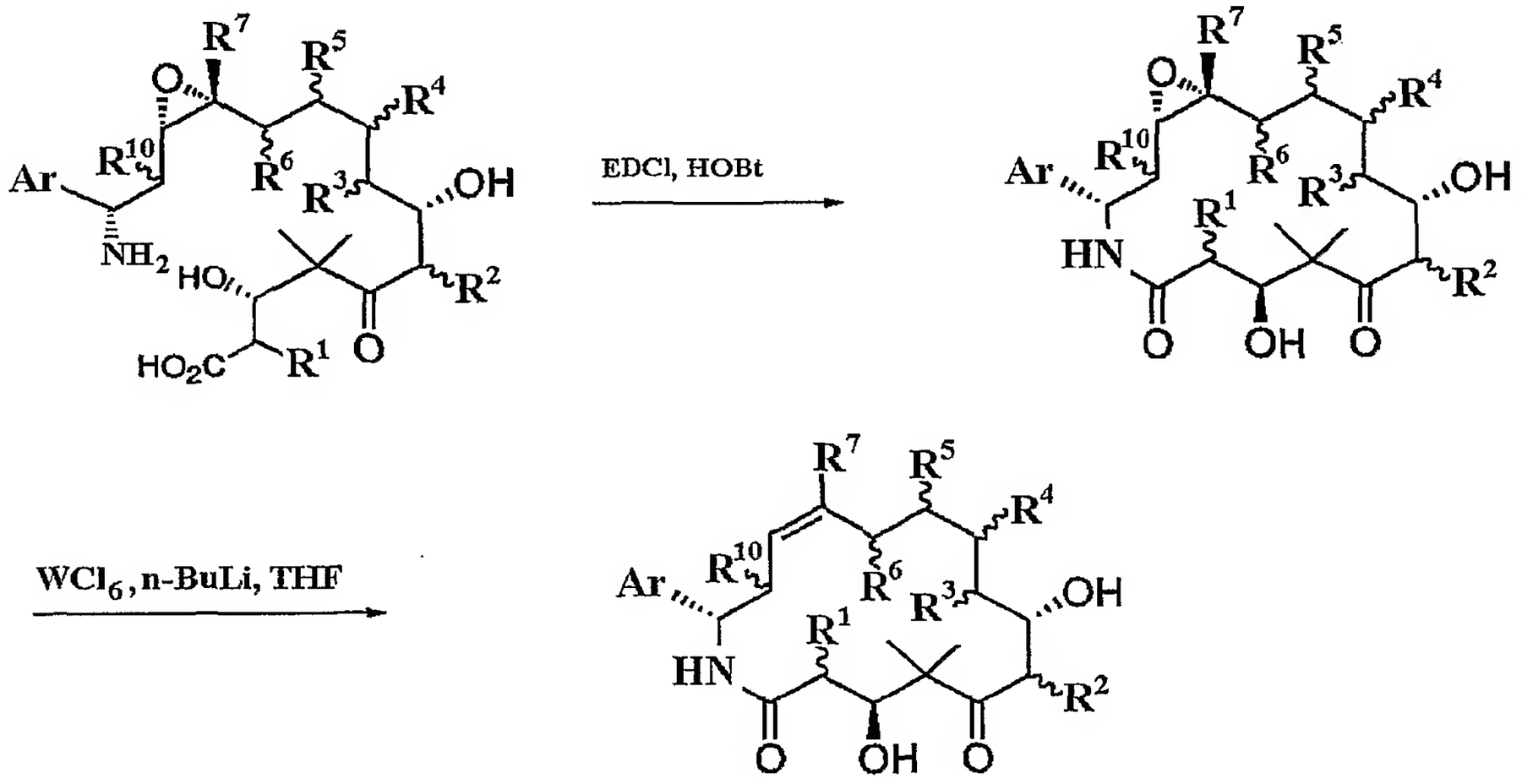
SCHEME 13



The oxy-macrolactone is treated with sodium azide and tetrakis(triphenylphosline) palladium to open the ring and form the azido acid. The azide is then reduced with trimethylphosphine to form the amino acid.

Epoxy-compounds where W is NH can be made from the macrolactamization of the amino acid.

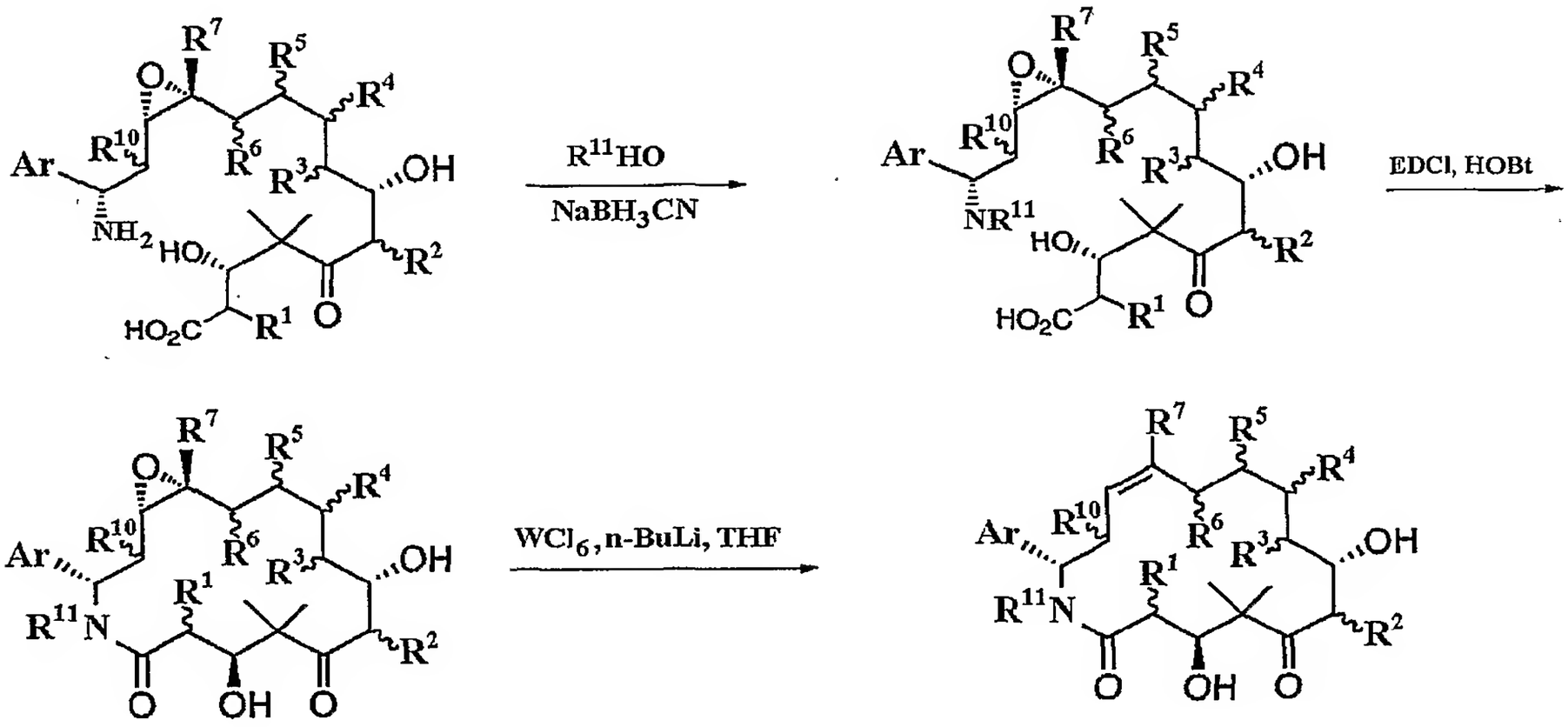
SCHEME 14



As shown by Scheme 14, the amino carboxy acid is treated with 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide and 1-hydroxybenzotriazole to form the epoxy-macrolactam. The desoxy-macrolactam can be made by treating the epoxy-macrolactam with tungsten hexachloride and butyllithium.

Epoxy-compounds where W is NR^{11} and R^{11} is not hydrogen can be made by treating the amino carboxy acid with an aldehyde and sodium cyanoborohydride prior to macrolactamization.

SCHEME 15



As shown by Scheme 15, the amino carboxy acid is treated with aldehyde, $R^{11}HO$, and sodium cyanoborohydride to form a substituted amino carboxy acid which is then macrolactamized. Alternatively, the amino carboxy acid is treated with ketone, R^{11}=O, and sodium cyanoborohydride to form a substituted amino carboxy acid which is then macrolactamized. Optionally, the epoxy compounds can deoxygenated as described previously (see e.g., second reaction of Scheme 14). These methods together provide the epoxy and desoxy macrolactams where R^{11} is not hydrogen.

The synthetic methods for making the macrolactams of the invention are also described in greater detail by the Examples 27-31. Example 27 describes the formation of the amino acid using 9-oxo-epothilone D as an illustrative starting material. Examples 28 and 29 describe the formation of the epoxy and desoxy macrolactam versions of 9-oxo-epothilone D respectively. Examples 30 and 31 describe the formation of the epoxy and desoxy substituted macrolactam versions of 9-oxo-epothilone D respectively.

Formulation

A composition of the present invention generally comprises an inventive compound and a pharmaceutically acceptable carrier. The inventive compound may be free form or

where appropriate as pharmaceutically acceptable derivatives such as prodrugs, and salts and esters of the inventive compound.

The composition may be in any suitable form such as solid, semisolid, or liquid form. See Pharmaceutical Dosage Forms and Drug Delivery Systems, 5th edition, Lippicott Williams & Wilkins (1991) which is incorporated herein by reference. In general, the pharmaceutical preparation will contain one or more of the compounds of the invention as an active ingredient in admixture with an organic or inorganic carrier or excipient suitable for external, enteral, or parenteral application. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, pessaries, solutions, emulsions, suspensions, and any other form suitable for use. The carriers that can be used include water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, and other carriers suitable for use in manufacturing preparations, in solid, semi-solid, or liquified form. In addition, auxiliary stabilizing, thickening, and coloring agents and perfumes may be used.

In one embodiment, the compositions containing an inventive compound are Cremophor®-free. Cremophor® (BASF Aktiengesellschaft) is a polyethoxylated castor oil which is typically used as a surfactant in formulating low soluble drugs. However, because Cremophor® can case allergic reactions in a subject, compositions that minimize or eliminate Cremophor® are preferred. Formulations of epothilone A or B that eliminate Cremophor® are described for example by PCT Publication WO 99/39694 which is incorporated herein by reference and may be adapted for use with the inventive compounds.

Where applicable, the inventive compounds may be formulated as microcapsules and nanoparticles. General protocols are described for example, by Microcapsules and Nanoparticles in Medicine and Pharmacy by Max Donbrow, ed., CRC Press (1992) and by U.S. Patent Nos. 5,510,118; 5,534,270; and 5,662,883 which are all incorporated herein by reference. By increasing the ratio of surface area to volume, these formulations allow for the oral delivery of compounds that would not otherwise be amenable to oral delivery.

The inventive compounds may also be formulated using other methods that have been previously used for low solubility drugs. For example, the compounds may form emulsions with vitamin E or a PEGylated derivative thereof as described by WO 98/30205 and 00/71163 which are incorporated herein by reference. Typically, the inventive compound is dissolved in an aqueous solution containing ethanol (preferably less than 1% w/v). Vitamin E or a PEGylated-vitamin E is added. The ethanol is then removed to form a pre-emulsion that can be formulated for intravenous or oral routes of administration. Another strategy involves encapsulating the inventive compounds in liposomes. Methods for forming liposomes as drug delivery vehicles are well known in the art. Suitable protocols include those described by U.S. Patent Nos. 5,683,715; 5,415,869, and 5,424,073 which are incorporated herein by reference relating to another relatively low solubility cancer drug taxol and by PCT Publication WO 01/10412 which is incorporated herein by reference relating to epothilone B. Of the various lipids that may be used, particularly preferred lipids for making epothilone-encapsulated liposomes include phosphatidylcholine and polyethyleneglycol-derivitized distearyl phosphatidylethanolamine. Example 32 provides an illustrative protocol for making liposomes containing 9-oxo-epothilone D, the general method which can be readily adapted to make liposomes containing other compounds of the present invention.

Yet another method involves formulating the inventive compounds using polymers such as polymers such as biopolymers or biocompatible (synthetic or naturally occurring) polymers. Biocompatible polymers can be categorized as biodegradable and non-biodegradable. Biodegradable polymers degrade *in vivo* as a function of chemical composition, method of manufacture, and implant structure. Illustrative examples of synthetic polymers include polyanhydrides, polyhydroxyacids such as polylactic acid, polyglycolic acids and copolymers thereof, polyesters polyamides polyorthoesters and some polyphosphazenes. Illustrative examples of naturally occurring polymers include proteins and polysaccharides such as collagen, hyaluronic acid, albumin, and gelatin.

Another method involves conjugating the compounds of the present invention to a polymer that enhances aqueous solubility. Examples of suitable polymers include polyethylene glycol, poly-(d-glutamic acid), poly-(l-glutamic acid), poly-(l-glutamic acid), poly-(d-aspartic acid), poly-(l-aspartic acid), poly-(l-aspartic acid) and copolymers thereof.

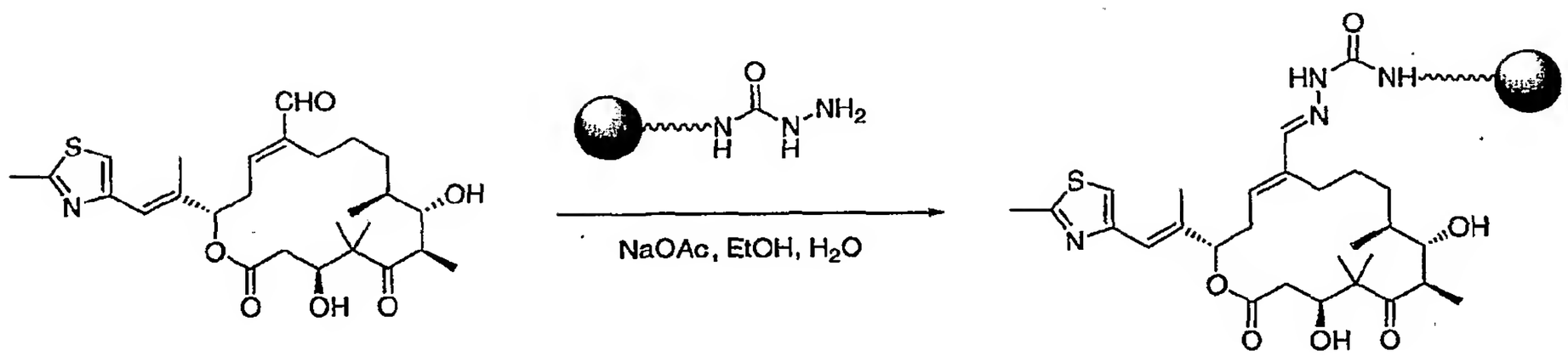
Polyglutamic acids having molecular weights between about 5,000 to about 100,000 are preferred, with molecular weights between about 20,000 and 80,000 being more preferred and with molecular weights between about 30,000 and 60,000 being most preferred. The polymer is conjugated via an ester linkage to one or more hydroxyls of an inventive epothilone using a protocol as essentially described by U.S. Patent No. 5,977,163 which is incorporated herein by reference, and by Example 33. Preferred conjugation sites include the hydroxyl off carbon-21 in the case of 21-hydroxy-derivatives of the present invention. Other conjugation sites include the hydroxyl off carbon 3, the hydroxyl off carbon 7 and where applicable, the hydroxyl off carbon 11.

In another method, the inventive compounds are conjugated to a monoclonal antibody. This strategy allows the targeting of the inventive compounds to specific targets. General protocols for the design and use of conjugated antibodies are described in Monoclonal Antibody-Based Therapy of Cancer by Michael L. Grossbard, ed. (1998) which is incorporated herein by reference.

In one embodiment, the compounds of the present invention include a semicarbazide linker which can then be conjugated to targets of interest, including antibodies. The semicarbazide linker is formed by condensing a carbonyl of an inventive compound with a hydrazine derivative. Suitable carbonyl groups include those off carbon-9 (e.g., 9-oxo-epothilone derivatives such as 9-oxo-epothilone D), C-21 (e.g., 21-oxo-epothilone derivatives such as 21-oxo-epothilone D), and C-26 (e.g., 26-oxo-epothilone derivatives such as 26-oxo-epothilone D).

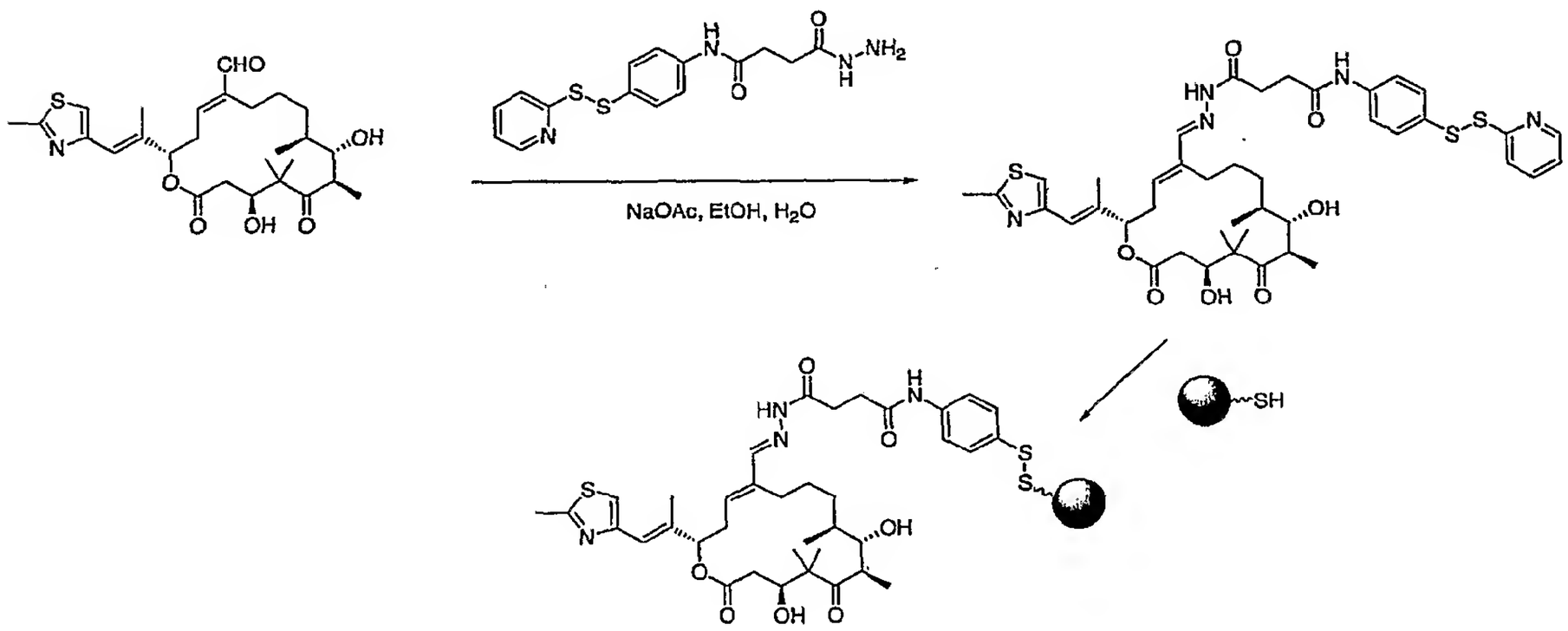
Scheme 16A illustrates one embodiment of a semicarbazide linker using 26-oxo-epothilone D as an example.

SCHEME 16A



Scheme 16B illustrates another embodiment of a specific semicarbazide linker using 26-oxo-epothilone D as an example.

SCHEME 16B



As illustrated by Scheme 16B, the semicarbazone-linked epothilone is made and then attached to a target of interest such as an antibody using disulfide exchange.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the subject treated and the particular mode of administration. For example, a formulation for intravenous use comprises an amount of the inventive compound ranging from about 1 mg/mL to about 25 mg/mL, preferably from about 5 mg/mL to 15 mg/mL, and more preferably about 10

mg/mL. Intravenous formulations are typically diluted between about 2 fold and about 30 fold with normal saline or 5% dextrose solution prior to use.

Methods to Treat Cancer

In one aspect of the present invention, the inventive compounds are used to treat cancer. In one embodiment, the compounds of the present invention are used to treat cancers of the head and neck which include tumors of the head, neck, nasal cavity, paranasal sinuses, nasopharynx, oral cavity, oropharynx, larynx, hypopharynx, salivary glands, and paragangliomas. In another embodiment, the compounds of the present invention are used to treat cancers of the liver and biliary tree, particularly hepatocellular carcinoma. In another embodiment, the compounds of the present invention are used to treat intestinal cancers, particularly colorectal cancer. In another embodiment, the compounds of the present invention are used to treat ovarian cancer. In another embodiment, the compounds of the present invention are used to treat small cell and non-small cell lung cancer. In another embodiment, the compounds of the present invention are used to treat breast cancer. In another embodiment, the compounds of the present invention are used to treat sarcomas which includes fibrosarcoma, malignant fibrous histiocytoma, embryonal rhabdomysocarcoma, leiomysosarcoma, neurofibrosarcoma, osteosarcoma, synovial sarcoma, liposarcoma, and alveolar soft part sarcoma. In another embodiment, the compounds of the present invention are used to treat neoplasms of the central nervous systems, particularly brain cancer. In another embodiment, the compounds of the present invention are used to treat lymphomas which include Hodgkin's lymphoma, lymphoplasmacytoid lymphoma, follicular lymphoma, mucosa-associated lymphoid tissue lymphoma, mantle cell lymphoma, B-lineage large cell lymphoma, Burkitt's lymphoma, and T-cell anaplastic large cell lymphoma.

The method comprises administering a therapeutically effective amount of an inventive compound to a subject suffering from cancer. The method may be repeated as necessary either to contain (i.e. prevent further growth) or to eliminate the cancer. Clinically, practice of the method will result in a reduction in the size or number of the cancerous growth and/ or a reduction in associated symptoms (where applicable).

Pathologically, practice of the method will produce at least one of the following: inhibition of cancer cell proliferation, reduction in the size of the cancer or tumor, prevention of further metastasis, and inhibition of tumor angiogenesis.

The compounds and compositions of the present invention can be used in combination therapies. In other words, the inventive compounds and compositions can be administered concurrently with, prior to, or subsequent to one or more other desired therapeutic or medical procedures. The particular combination of therapies and procedures in the combination regimen will take into account compatibility of the therapies and/or procedures and the desired therapeutic effect to be achieved.

In one embodiment, the compounds and compositions of the present invention are used in combination with another anti-cancer agent or procedure. Illustrative examples of other anti-cancer agents include but are not limited to: (i) alkylating drugs such as mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide; (ii) antimetabolites such as methotrexate; (iii) microtubule stabilizing agents such as vinblastin, paclitaxel, docetaxel, and discodermolide; (iv) angiogenesis inhibitors; (v) and cytotoxic antibiotics such as doxorubicon (adriamycin), bleomycin, and mitomycin. Illustrative examples of other anti-cancer procedures include: (i) surgery; (ii) radiotherapy; and (iii) photodynamic therapy.

In another embodiment, the compounds and compositions of the present invention are used in combination with an agent or procedure to mitigate potential side effects from the inventive compound or composition such as diarrhea, nausea and vomiting. Diarrhea may be treated with antidiarrheal agents such as opioids (e.g. codeine, diphenoxylate, difenoxin, and loeramide), bismuth subsalicylate, and octreotide. Nausea and vomiting may be treated with antiemetic agents such as dexamethasone, metoclopramide, diphenyhydramine, lorazepam, ondansetron, prochlorperazine, thiethylperazine, and dronabinol. For those compositions that includes polyethoxylated castor oil such as Cremophor®, pretreatment with corticosteroids such as dexamethasone and methylprednisolone and/or H_1 antagonists such as diphenylhydramine HCl and/or H_2 antagonists may be used to mitigate anaphylaxis. Illustrative formulations for intravenous use and pretreatment regiments are described by Examples 35 and 36 respectively.

Methods of Treating of Non-cancer, Cellular Hyperproliferative Disorders

In another aspect of the present invention, the inventive compounds are used to treat non-cancer disorders that are characterized by cellular hyperproliferation. Illustrative examples of such disorders include but are not limited to: atrophic gastritis, inflammatory hemolytic anemia, graft rejection, inflammatory neutropenia, bullous pemphigoid, coeliac disease, demyelinating neuropathies, dermatomyositis, inflammatory bowel disease (ulcerative colitis and Crohn's disease), multiple sclerosis, myocarditis, myositis, nasal polyps, chronic sinusitis, pemphigus vulgaris, primary glomerulonephritis, psoriasis, surgical adhesions, stenosis or restenosis, scleritis, scleroderma, eczema (including atopic dermatitis. irritant dermatitis, allergic dermatitis), periodontal disease (i.e., periodontitis), polycystic kidney disease, and type I diabetes.

Other examples include vasculitis (e.g., Giant cell arteritis (temporal arteritis, Takayasu's arteritis), polyarteritis nodosa, allergic angiitis and granulomatosis (Churg-Strauss disease), polyangitis overlap syndrome, hypersensitivity vasculitis (Henoch-Schonlein purpura), serum sickness, drug- induced vasculitis, infectious vasculitis, neoplastic vasculitis, vasculitis associated with connective tissue disorders, vasculitis associated with congenital deficiencies of the complement system, Wegener's granulomatosis, Kawasaki's disease, vasculitis of the central nervous system, Buerger's disease and systemic sclerosis); gastrointestinal tract diseases (e.g., pancreatitis, Crohn's disease, ulcerative colitis, ulcerative proctitis, primary sclerosing cholangitis, benign strictures of any cause including ideopathic (e.g., strictures of bile ducts, esophagus, duodenum, small bowel or colon); respiratory tract diseases (e.g., asthma, hypersensitivity pneumonitis, asbestosis, silicosis and other forms of pneumoconiosis, chronic bronchitis and chronic obstructive airway disease); nasolacrimal duct diseases (e.g., strictures of all causes including ideopathic); and eustachean tube diseases (e.g., strictures of all causes including ideopathic).

The method of treating such diseases comprises administering a therapeutically effective amount of an inventive compound to a subject suffering therefrom. The method may be repeated as necessary. The inventive methods are described in greater detail below with reference to three illustrative non-cancer disorders.

In one embodiment, the compounds of the present invention are used to treat psoriasis, a condition characterized by the cellular hyperproliferation of keratinocytes which builds up on the skin to form elevated, scaly lesions. The method comprises administering a therapeutically effective amount of an inventive compound to a subject suffering from psoriasis. The method may be repeated as necessary either to decrease the number or severity of lesions or to eliminate the lesions. Clinically, practice of the method will result in a reduction in the size or number of skin lesions, diminution of cutaneous symptoms (pain, burning and bleeding of the affected skin) and/ or a reduction in associated symptoms (e.g., joint redness, heat, swelling, diarrhea. abdominal pain). Pathologically, practice of the method will result in at least one of the following: inhibition of keratinocyte proliferation, reduction of skin inflammation (for example, by impacting on: attraction and growth factors, antigen presentation, production of reactive oxygen species and matrix metalloproteinases), and inhibition of dermal angiogenesis.

In another embodiment, the compounds of the present invention are used to treat multiple sclerosis, a condition characterized by progressive demyelination in the brain. Although the exact mechanisms involved in the loss of myelin are not understood, there is an increase in astrocyte proliferation and accumulation in the areas of myelin destruction. At these sites, there is macrophage-like activity and increased protease activity which is at least partially responsible for degradation of the myelin sheath. The method comprises administering a therapeutically effective amount of an inventive compound to a subject suffering from multiple sclerosis. The method may be repeated as necessary to inhibit astrocyte proliferation and/or lessen the severity of the loss of motor function and/or prevent or attenuate chronic progression of the disease. Clinically, practice of the method will result in improvement in visual symptoms (visual loss, diplopia), gait disorders (weakness, axial instability, sensory loss, spasticity, hyperreflexia, loss of dexterity), upper extremity dysfunction (weakness, spasticity, sensory loss), bladder dysfunction (urgency, incontinence, hesitancy, incomplete emptying), depression, emotional lability, and cognitive impairment. Pathologically, practice of the method will result in the reduction of one or more of the following, such as myelin loss, breakdown of the blood-brain barrier, perivascular infiltration of mononuclear cells, immunologic abnormalities, gliotic scar

formation and astrocyte proliferation, metalloproteinase production, and impaired conduction velocity.

In another embodiment, the compounds of the present invention are used to treat rheumatoid arthritis, a multisystem chronic, relapsing, inflammatory disease that sometimes leads to destruction and ankyiosis of affected joints. Rheumatoid arthritis is characterized by a marked thickening of the synovial membrane which forms villous projections that extend into the joint space, multilayering of the synoviocyte lining (synoviocyte proliferation), infiltration of the synovial membrane with white blood cells (macrophages, lymphocytes, plasma cells, and lymphoid follicles; called an "inflammatory synovitis"), and deposition of fibrin with cellular necrosis within the synovium. The tissue formed as a result of this process is called pannus and, eventually the pannus grows to fill the joint space. The pannus develops an extensive network of new blood vessels through the process of angiogenesis that is essential to the evolution of the synovitis. Release of digestive enzymes (matrix metalloproteinases (e.g., collagenase, stromelysin)) and other mediators of the inflammatory process (e.g., hydrogen peroxide, superoxides, lysosomal enzymes, and products of arachadonic acid metabolism) from the cells of the pannus tissue leads to the progressive destruction of the cartilage tissue. The pannus invades the articular cartilage leading to erosions and fragmentation of the cartilage tissue. Eventually there is erosion of the subchondral bone with fibrous ankylosis and ultimately bony ankylosis, of the involved joint.

The method comprises administering a therapeutically effective amount of an inventive compound to a subject suffering from rheumatoid arthritis. The method may be repeated as necessary to accomplish to inhibit synoviocyte proliferation and/or lessen the severity of the loss of movement of the affected joints and/or prevent or attenuate chronic progression of the disease. Clinically, practice of the present invention will result in one or more of the following: (i) decrease in the severity of symptoms (pain, swelling and tenderness of affected joints; morning stiffness. weakness, fatigue. anorexia, weight loss); (ii) decrease in the severity of clinical signs of the disease (thickening of the joint capsule. synovial hypertrophy, joint effusion, soft tissue contractures, decreased range of motion, ankylosis and fixed joint deformity); (iii) decrease in the extra-articular manifestations of the disease (rheumatic nodules, vasculitis, pulmonary nodules, interstitial fibrosis,

pericarditis, episcleritis, iritis, Felty's syndrome, osteoporosis); (iv) increase in the frequency and duration of disease remission/ symptom-free periods; (v) prevention of fixed impairment and disability; and/ or (vi) prevention/attenuation of chronic progression of the disease. Pathologically, practice of the present invention will produce at least one of the following: (i) decrease in the inflammatory response; (ii) disruption of the activity of inflammatory cytokines (such as IL-I, TNFa, FGF, VEGF); (iii) inhibition of synoviocyte proliferation; (iv) inhibition of matrix metalloproteinase activity, and/ or (v) inhibition of angiogenesis.

In another embodiment, the compounds of the present invention are used to threat atherosclerosis and/or restenosis, particularly in patients whose blockages may be treated with an endovascular stent. Atheroschlerosis is a chronic vascular injury in which some of the normal vascular smooth muscle cells ("VSMC") in the artery wall, which ordinarily control vascular tone regulating blood flow, change their nature and develop "cancer-like" behavior. These VSMC become abnormally proliferative, secreting substances (growth factors, tissue-degradation enzymes and other proteins) which enable them to invade and spread into the inner vessel lining, blocking blood flow and making that vessel abnormally susceptible to being completely blocked by local blood clotting. Restenosis, the recurrence of stenosis or artery stricture after corrective procedures, is an accelerated form of atherosclerosis.

The method comprises coating a therapeutically effective amount of an inventive compound on a stent and delivering the stent to the diseased artery in a subject suffering from atherosclerosis. Methods for coating a stent with a compound are described for example by U.S. Patent Nos. 6,156,373 and 6,120, 847. Clinically, practice of the present invention will result in one or more of the following: (i) increased arterial blood flow; (ii) decrease in the severity of clinical signs of the disease; (iii) decrease in the rate of restenosis; or (iv) prevention/attenuation of the chronic progression of atherosclerosis. Pathologically, practice of the present invention will produce at least one of the following at the site of stent implanataion: (i) decrease in the inflammatory response, (ii) inhibition of VSMC secretion of matrix metalloproteinases; (iii) inhibition of smooth muscle cell accumulation; and (iv) inhibition of VSMC phenotypic dedifferentiation.

Dosage Levels

In one embodiment, dosage levels that are administered to a subject suffering from cancer or a non-cancer disorder characterized by cellular proliferation are of the order from about 1 mg/m^2 to about 200 mg/m^2 which may be administered as a bolus (in any suitable route of administration) or a continuous infusion (e.g. 1 hour, 3 hours, 6 hours, 24 hours, 48 hours or 72 hours) every week, every two weeks, or every three weeks as needed. It will be understood, however, that the specific dose level for any particular patient depends on a variety of factors. These factors include the activity of the specific compound employed; the age, body weight, general health, sex, and diet of the subject; the time and route of administration and the rate of excretion of the drug; whether a drug combination is employed in the treatment; and the severity of the condition being treated.

In another embodiment, the dosage levels are from about 10 mg/m^2 to about 150 mg/m^2, preferably from about 10 to about 75 mg/m^2 and more preferably from about 15 mg/m^2 to about 50 mg/m^2 once every three weeks as needed and as tolerated. In another embodiment, the dosage levels are from about 1 mg to about 150 mg/m^2, preferably from about 10 mg/m^2 to about 75 mg/m^2 and more preferably from about 25 mg/m^2 to about 50 mg/m^2 once every two weeks as needed and as tolerated. In another embodiment, the dosage levels are from about 1 mg/m^2 to about 100 mg/m^2, preferably from about 5 mg/m^2 to about 50 mg/m^2 and more preferably from about 10 mg/m^2 to about 25 mg/m^2 once every week as needed and as tolerated. In another embodiment, the dosage levels are from about 0.1 to about 25 mg/m^2, preferably from about 0.5 to about 15 mg/m^2 and more preferably from about 1 mg/m^2 to about 10 mg/m^2 once daily as needed and tolerated.

A detailed description of the invention having been provided above, the following examples are given for the purpose of illustrating the present invention and shall not be construed as being a limitation on the scope of the invention or claims.

EXAMPLE 1

Biological Activity

Compounds of the invention are screened for anticancer activity in four different human tumor cell lines (MCF-7 (breast), NCI/ADR-Res (breast, MDR), SF-268 (glioma), NCI-H460 (lung)) using sulforhodamine B (SRB) assay. The cells were maintained in a 5% CO2-humidified atmosphere at 37 degree in RPMI 1640 medium (Life Technology) supplemented with 10% fetal bovine serum (Hyclone) and 2mM L-glutamine.

Cytotoxicity of the inventive compounds is determined by SRB assay (Skehan *et al., J. Natl. Cancer Inst.* **82**: 1107-1112 (1990) which is incorporated herein by reference). Cultured cells are trypsinized, counted and diluted to the following concentrations per 100 μl with growth medium: MCF-7, 5000; NCI/ADR-Res, 7500; NCI-H460, 5000; and, SF-268, 7500. The cells are seeded at 100 μl/well in 96-well microtiter plates. Twenty hours later, 100 μl of a compound of interest (ranging from 1000 nM to 0.001 nM diluted in growth medium) is added to each well. After incubation with the compound for 3 days, the cells are fixed with 100 μl of 10% trichloric acid ("TCA") at 4 degree for 1 hour, and are stained with 0.2% SRB/1% acetic acid at room temperature for 20 minutes. The unbound dye is rinsed away with 1% acetic acid, and the bound SRB is then extracted by 200 μl of 10 mM Tris base. The amount of bound dye is determined by OD 515 nm, which correlates with the total cellular protein contents. The data is then analyzed using Kaleida Graph program and the IC_{50}'s calculated.

For tubulin polymerization assays, MCF-7 cells are grown to confluency in 35 mm-culture dishes and treated with 1 μM of a compound of interest for 0, 1 or 2 hours at 37 degree (Giannakakou et al., *J. Biol. Chem.* **271**:17118-17125 (1997); *Int. J. Cancer* **75**: 57-63 (1998) which are incorporated herein by reference). After washing the cells twice with 2 ml of PBS without calcium or magnesium, the cells are lysed at room temperature for 5-10 minutes with 300 μl of lysis buffer (20 mM Tris, PH 6.8, 1 mM $MgCl_2$, 2 mM EGTA, 1% Triton X-100, plus protease inhibitors). The cells are scraped and the lysates are transferred to 1.5-ml Eppendof tubes. The lysates are then centrifuged at 18000 g for 12 minutes at room temperature. The supernatant containing soluble or unpolymerized

(cytosolic) tubulin are separated from pellets containing insoluble or polymerized (cytoskeletal) tubulin and transferred to new tubes. The pellets are then resuspended in 300 μl of lysis buffer. Changes in tubulin polymerization in the cell are determined by analyzing same volume of aliquots of each sample with SDS-PAGE, followed by immunoblotting using an anti-tubulin antibody (Sigma).

EXAMPLE 2

Hydroxylation of epothilone D

A mixture of selenium dioxide (50 mg), 0.4 mL of *tert*-butylhydroperoxide (5-6 M solution in decane), 0.5 mL of dichloromethane, and 0.35 mL of water was stirred at room temperature for 15 minutes. A solution of epothilone D (200 mg) in 1.5 mL of CH_2Cl_2 was added and the mixture was stirred for 48 hours. The mixture was diluted with 20 mL of CH_2Cl_2 and shaken with 10 mL of sat. aq. $NaHCO_3$. The phases were separated, and the organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in 2 mL of CH_2Cl_2 and chromatographed on a 35-gm ISCO silica column equilibrated in 80:20 ethyl acetate/hexanes at a flow rate of 20 mL/min. After 20 minutes, the solvent mixture was ramped to 100% ethyl acetate over a 10 minute period. Products eluted in the following order: (a) unreacted epothilone D (47 mg); (b) mixed carbonyl compounds, including 11-oxoepothilone D and 26-oxoepothilone D (18 mg); (c) diastereomer B of 11-hydroxyepothilone D (19 mg); (d) diastereomer A of 11-hydroxyepothilone D (33 mg); and 26-hydroxyepothilone D (45 mg).

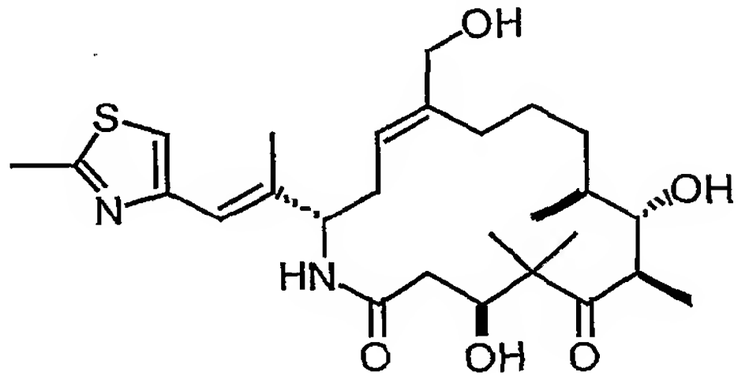
11-hydroxyepothilone D (diastereomer A): ^{13}C-NMR ($CDCl_3$): δ 220.3, 170.5, 165.0, 152.0, 139.5, 137.2, 122.0, 119.8, 116.0, 78.1, 77.3, 75.3, 71.9, 53.0, 42.2, 38.8, 38.1, 31.7, 31.5, 27.8, 21.5, 19.9, 19.0, 15.7, 15.0, 13.9, 11.5.

11-hydroxyepothilone D (diastereomer B): ^{13}C-NMR ($CDCl_3$): δ 220.0, 170.2, 165.1, 151.9, 140.2, 136.5, 120.9, 119.0, 115.3, 78.8, 77.3, 76.4, 71.5, 52.9, 43.2, 39.3, 36.9, 32.7, 29.6, 28.1, 20.9, 19.7, 18.9, 16.9, 16.5, 14.9, 10.9.

26-hydroxyepothilone D: ^{13}C-NMR ($CDCl_3$): δ 220.6, 170.2, 165.1, 151.8, 141.8, 138.6, 121.6, 119.1, 115.5, 78.1, 74.0, 71.8, 66.1, 53.7, 41.6, 39.6, 37.9, 31.8, 31.6, 27.9, 25.2, 22.8, 18.9, 17.6, 16.0, 15.8, 13.3.

EXAMPLE 3

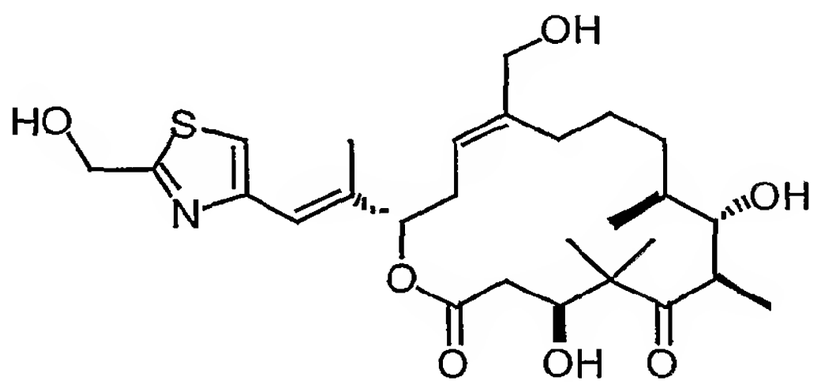
26-hydroxy-epothilone D lactam



Epothilone D lactam is treated with selenium dioxide according to Example 2. The hydroxylated products are separated by silica gel chromatography.

EXAMPLE 4

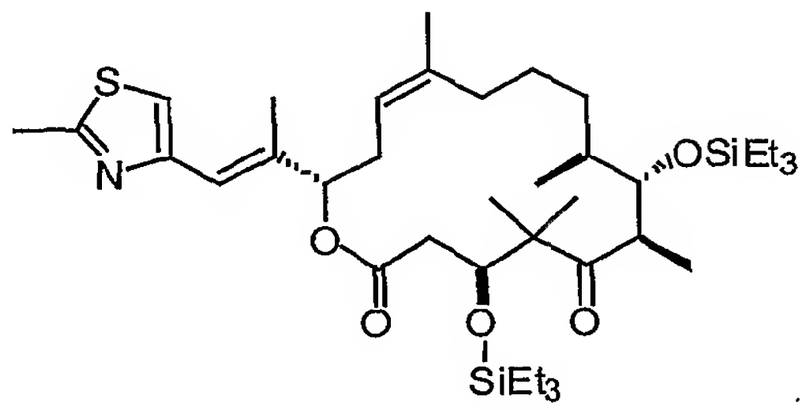
21,26-dihydroxy-epothilone D



21-hydroxy-epothilone D is treated with selenium dioxide according to Example 2. The hydroxylated products are separated by silica gel chromatography.

EXAMPLE 5

3,7-bis(O-triethylsilyl)epothilone D



A mixture of epothilone D (50 mg) in CH_2Cl_2 (5 mL), chlorotriethylsilane (100 uL), and 4-dimethylaminopyridine (50 mg) was stirred 12 hours at ambient temperature. The mixture was diluted with ether (25 mL) and washed successively with water, 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in hexanes and chromatographed on SiO_2 (hexanes followed by 3:1 hexanes/ether) to yield 62 mg (85%) of product. ^{13}C-NMR ($CDCl_3$): δ 215.3, 171.0, 164.5, 152.6, 140.5, 138.7, 119.6, 119.2, 116.1, 79.84, 79.83, 76.1, 53.5, 48.0, 39.3, 37.3, 32.4, 32.0, 31.2, 27.4, 24.5, 23.7, 23.0, 19.2, 17.5, 15.0, 7.2 (3C), 6.9 (3C), 5.6 (3C), 5.2 (3C).

EXAMPLE 6

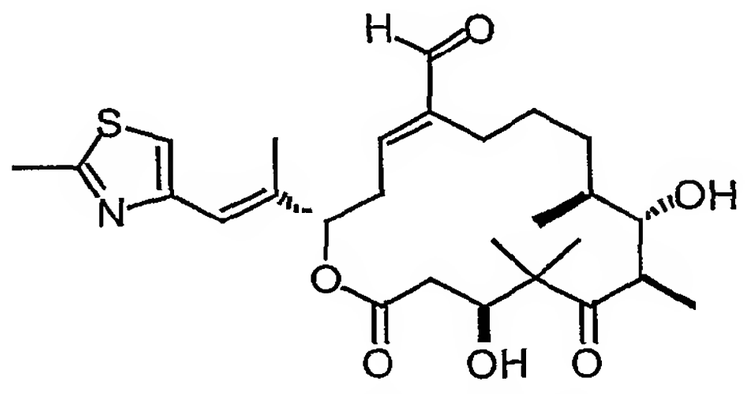
Hydroxylation of 3,7-bis(O-triethylsilyl)epothilone D

A mixture of selenium dioxide (15 mg), 0.1 mL of *tert*-butylhydroperoxide (5-6 M solution in decane), 0.2 mL of dichloromethane, and 0.1 mL of water was stirred at room temperature for 15 minutes. A solution of 3,7-bis(O-triethylsilyl)epothilone D (50 mg) (Example 4) in 0.5 mL of CH_2Cl_2 was added and the mixture was stirred for 24 hours. An additional 15 mg of selenium dioxide was added, and the reaction is continued an additional 24 hours. The mixture was diluted with 20 mL of CH_2Cl_2 and shaken with 10 mL of sat. aq. $NaHCO_3$. The phases were separated, and the organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in 2 mL of CH_2Cl_2 and chromatographed on a 35-gm ISCO silica column (gradient from 5% ethyl acetate/hexanes to 100% ethyl acetate). The products identified included the 3,7-bis(O-triethylsilyl)-

derivatives of the 26-oxo, 26-hydroxy, and 11-hydroxyepothilones D. Also identified was 3,7-bis(O-triethylsilyl)-11,26-dihydroxyepothilone D: ^{13}C-NMR (CDCl3): δ 215.1, 170.8, 164.8, 152.3, 143.7, 137.8, 124.5, 120.4, 116.6, 80.0, 79.0, 76.4, 70.7, 65.8, 53.4, 48.4, 39.3, 36.9, 35.0, 32.2, 28.2, 24.8, 23.9, 19.2 (2C), 17.7, 14.8, 7.2 (3C), 6.9 (3C), 5.6 (3C), 5.2 (3C).

EXAMPLE 7

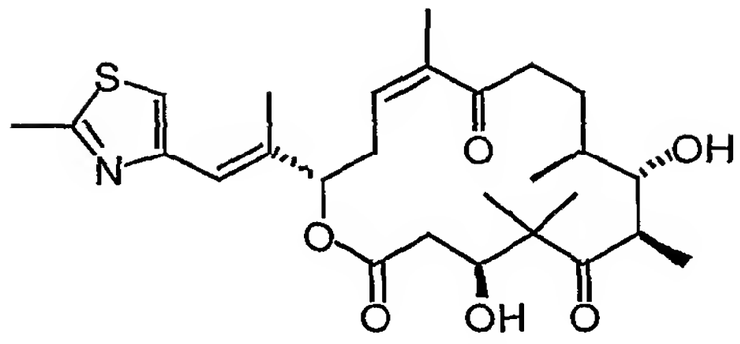
26-oxo-epothilone D



A suspension of 26-hydroxyepothilone D (4 mg) and activated manganese dioxide (16 mg) in 0.2 mL of CH_2Cl_2 was stirred for 1 hour at ambient temperature. The suspension was filtered through a 1-cm plug of silica gel using ethyl acetate, and the eluate was evaporated to yield the product as a clear glass. ^{1}H-NMR ($CDCl_3$): δ 9.39 (s,1H), 6.99 (s,1H), 6.45 (br s,1H), 6.45 (dd,1H,J=5.6,10.0 Hz), 5.41 (dd,1H,J=2.4,9.6 Hz), 4.30 (m,1H), 3.66 (m,1H), 3.50 (d,1H,J=6.4 Hz), 3.14 (dq,1H,J=4.4,6.8 Hz), 2.96 (dt,1H,J=9.6,15.2 Hz), 2.89 (d,1H,J=2.8 Hz), 2.70 (s,3H), 2.64 (ddd,1H,J=2.4,5.2,15.2 Hz), 2.46 (dd,1H,J=10.8,14.4 Hz), 2.42 (m,1H), 2.26 (dd,1H,J=2.4,14.4 Hz), 2.21 (dd,1H,J=6.8,12.8 Hz), 2.13 (d,3H,J=1.2 Hz), 1.70 (m,1H), 1.69 (d,3H,J=1.2 Hz), 1.36 (s,3H), 1.2-1.4 (m,4H), 1.17 (d,3H,J=6.8 Hz), 1.06 (s,3H), 1.00 (d,3H,J=6.8 Hz). ^{13}C-NMR ($CDCl_3$): δ 220.3, 194.7, 170.1, 165.4, 151.6, 148.6, 145.9, 137.9, 120.1, 116.2, 77.3, 73.6, 72.2, 53.6, 41.7, 39.6, 37.6, 33.4, 31.4, 25.5, 24.7, 22.9, 19.1, 17.6, 15.9 (2C), 13.0.

EXAMPLE 8

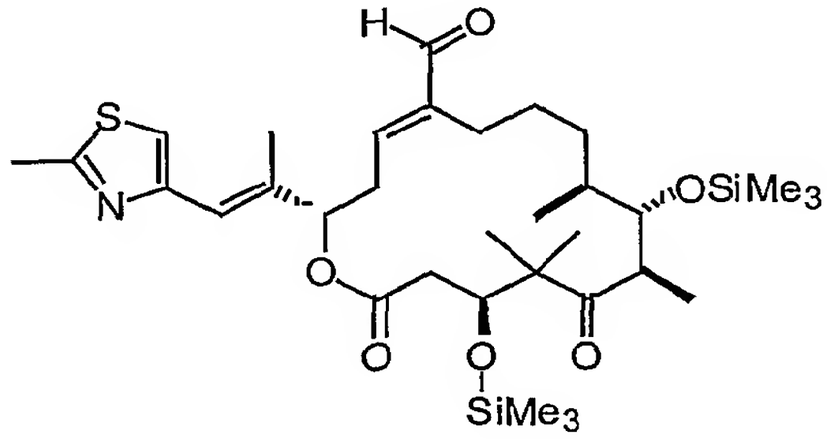
11-oxo-epothilone D



A suspension of 11-hydroxyepothilone D (4 mg) and activated manganese dioxide (16 mg) in 0.2 mL of CH_2Cl_2 was stirred for 3 hours at ambient temperature. The suspension was filtered through a 1-cm plug of silica gel using ethyl acetate, and the eluate was evaporated to yield the product as a clear glass. ^{1}H-NMR ($CDCl_3$): δ 6.96 (s,1H), 6.68 (br t,1H,J=5.6 Hz), 6.55 (br s,1H), 5.46 (dd,1H,J=4.0,8.4 Hz), 4.22 (ddd,1H,J=4.4, 5.6, 9.6 Hz), 3.67 (br q, J=5.2 Hz), 3.24 (m,1H), 2.96 (ddd,1H,J=7.6,7.6,14.4 Hz), 2.86 (d,1H,J=5.6 Hz), 2.65-2.80 (m,2H), 2.70 (s,3H), 2.47 (2H,m), 2.25 (ddd,1H,J=6.4,8.0,14.4 Hz), 2.11 (d,3H,J=1.2 Hz), 1.62-1.82 (m,2H), 1.77 (br s,3H), 1.34 (s,3H), 1.11 (d,3H,J=6.8), 1.05 (s,3H), 0.88 (d,3H,J=6.8).

EXAMPLE 9

3,7-bis(O-trimethylsilyl)-26-oxoepothilone D

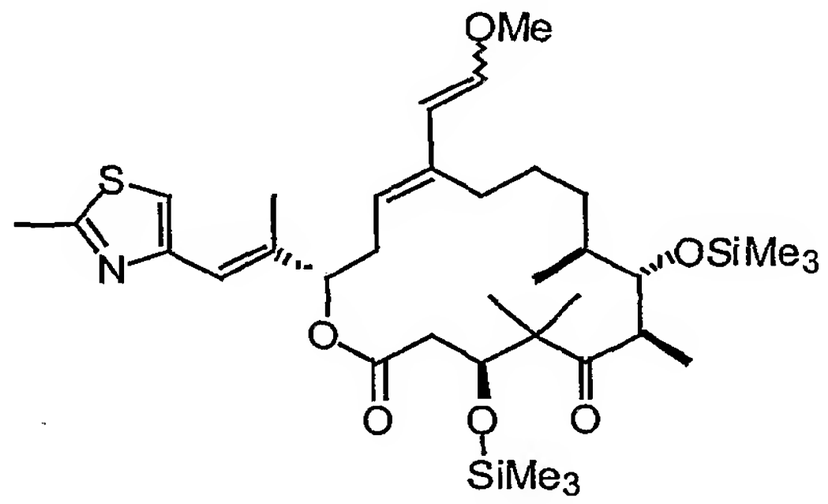


A solution of 26-oxoepothilone D (5.06 g) in 10 mL of CH_2Cl_2 is treated with chlorotrimethylsilane (5.0 mL) and 4-(dimethylamino)pyridine (3.7 g). After stirring for 12 hours, the mixture is diluted with ether and washed sequentially with water and sat.

$NaHCO_3$. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 10

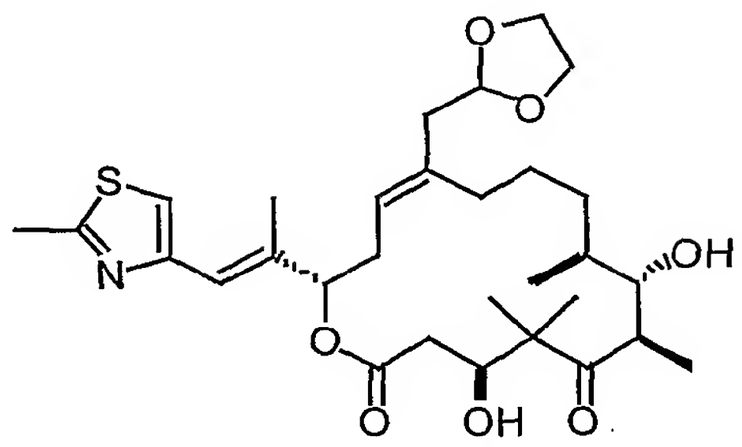
3,7-bis(O-trimethylsilyl)-26-methoxymethylidene-epothilone D



A suspension of vacuum-dried (methoxymethyl)triphenylphosphonium chloride (3.42 g) in 100 mL of THF under argon is treated with a 1.6 M solution of n-butyllithium in hexane (6.25 mL) over 30 minutes. After an additional 30 min, the solution is added dropwise to a solution of 3,7-bis(O-trimethylsilyl)-26-oxoepothilone D (6.50 g) at –30 °C. The reaction is continued at –30 °C for 1 hour, then allowed to warm to ambient temperature and continued for an additional 1 hour. The reaction is diluted with ether and quenched by addition of sat. NH_4Cl. The phases are separated, and the organic phase is washed sequentially with water and sat. $NaHCO_3$. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 11

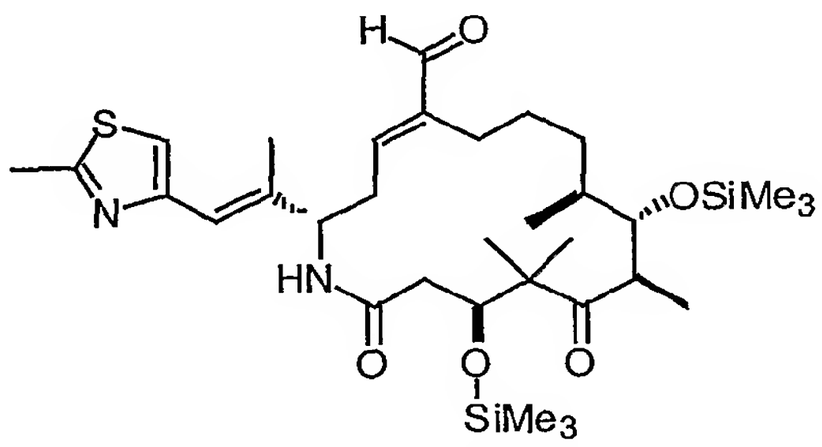
26-(2-dioxolanyl)-epothilone D



A solution of 3,7-bis(O-triethylsilyl)-26-methoxymethylidene-epothilone D (6.8 g), pyridinium p-toluenesulfonate (0.25 g), and 10 mL of ethylene glycol in 100 mL of THF is stirred at ambient temperature for 12 hours. The mixture is diluted with ethyl acetate and washed successively with water, sat. $NaHCO_3$, and brine. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 12

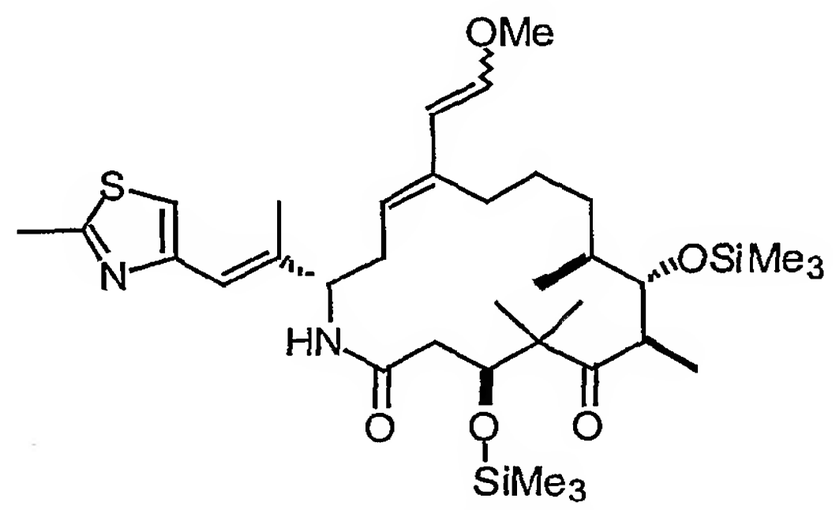
3,7-bis(O-trimethylsilyl)-26-oxoepothilone D lactam



A solution of 26-oxoepothilone D lactam (5.06 g) in 10 mL of CH_2Cl_2 is treated with chlorotrimethylsilane (5 mL) and (4-dimethylamino)pyridine (3.7 g). After stirring for 1 hour, the mixture is diluted with ether and washed sequentially with water and sat. $NaHCO_3$. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 13

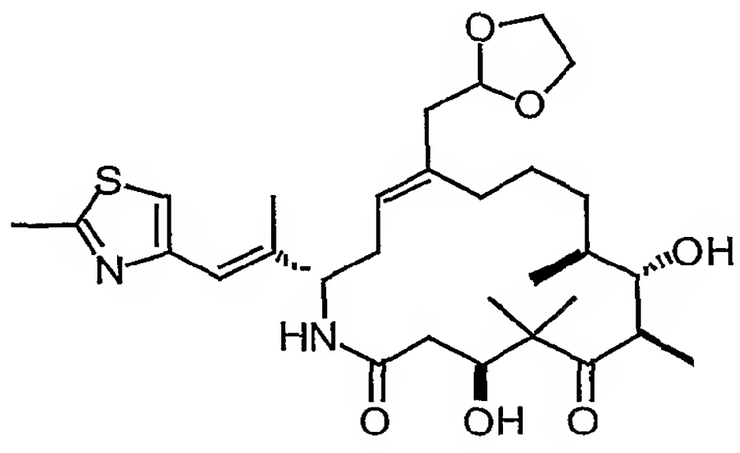
3,7-bis(O-trimethylsilyl)-26-methoxymethylidenyl-epothilone D lactam



A suspension of vacuum-dried (methoxymethyl)triphenylphosphonium chloride (3.42 g) in 100 mL of THF under argon is treated with a 1.6 M solution of n-butyllithium in hexane (6.25 mL) over 30 minutes. After an additional 30 min, the solution is added dropwise to a solution of 3,7-bis(O-trimethylsilyl)-26-oxoepothilone D lactam (6.0 g) at –30 °C. The reaction is continued at –30 °C for 1 hour, then allowed to warm to ambient temperature and continued for an additional 1 hour. The reaction is diluted with ether and quenched by addition of sat. NH_4Cl. The phases are separated, and the organic phase is washed sequentially with water and sat. $NaHCO_3$. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 14

26-(2-dioxolanyl)-epothilone D lactam

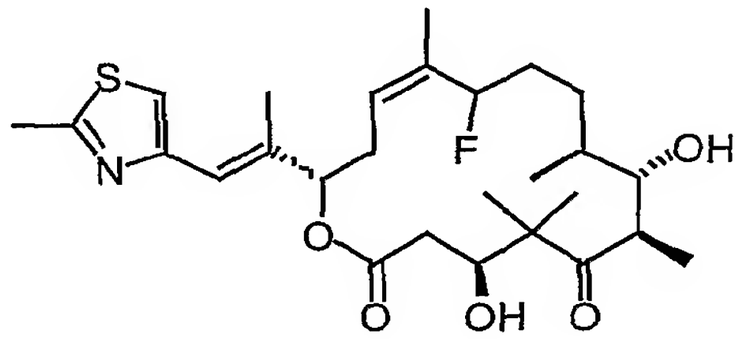


A solution of 3,7-bis(O-trimethylsilyl)-26-methoxymethylidenyl-epothilone D lactam (6.78 g), pyridinium p-toluenesulfonate (0.25 g), and 10 mL of ethylene glycol in

100 mL of THF is stirred at ambient temperature for 12 hours. The mixture is diluted with ethyl acetate and washed successively with water, sat. $NaHCO_3$, and brine. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 15

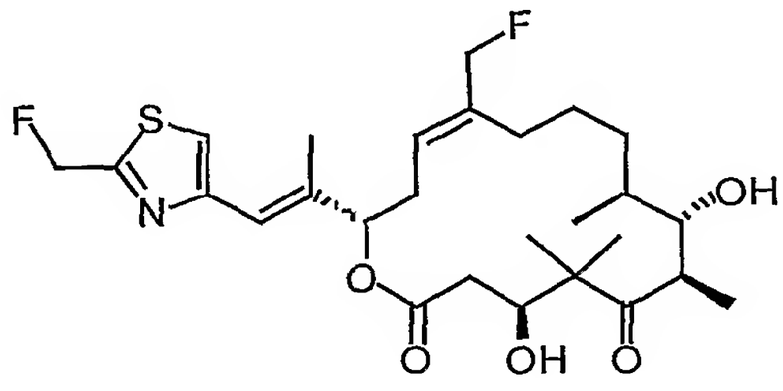
3,7-bis(O-triethylsilyl)-11-fluoro-epothilone D



A solution of 3,7-bis(O-triethylsilyl)-11-hydroxyepothilone D (7.36 g) in 100 mL of anhydrous CH_3CN is cooled to –78 °C and treated with pyridine (1.0 mL) and trifluoromethanesulfonic anhydride (1.8 mL). After 30 minutes, a 1.0 M solution of tetrabutylammonium fluoride in THF (50 mL) is added and the mixture is allowed to warm to ambient temperature. After 1 hour at ambient temperature, the mixture is diluted with ethyl acetate and washed successively with water, sat. $NaHCO_3$, and brine. The solution is dried over Na_2SO_4, filtered, and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 16

21,26-difluoro-epothilone D



Step 1. Preparation of 21,16-diiodoepothilone D

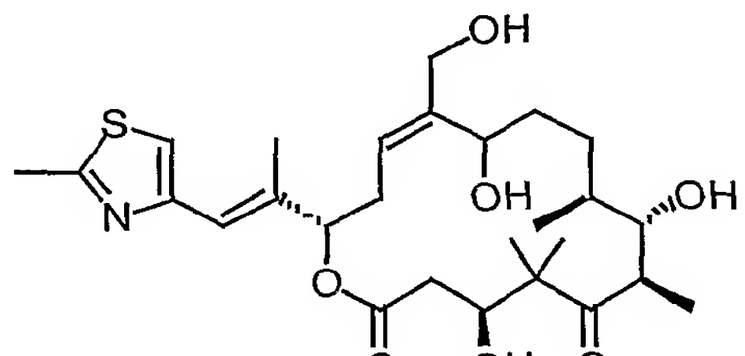
A solution of 21,16-dihydroxy-epothilone D (5.28 g) in 25 mL of acetonitrile and 50 mL of ether is treated with imidazole (2.1 g), triphenylphosphine (7.9 g), and iodine (7.6 g). After 1 hour, the mixture is diluted with 200 mL of ether, washed with sat. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The diiodide is isolated by flash chromatography on SiO_2, and is used immediately in the next step.

Step 2. Preparation of 21,16-difluoroepothilone D

A solution of the diiodide from Step 1 in 100 mL of acetonitrile is treated with tetrabutylammonium triphenyldifluorosilicate (22 g) at reflux for 12 hours. The mixture is concentrated, and the residue is diluted with ethyl acetate and washed successively with water, sat. $NaHCO_3$, and brine. After drying over $MgSO_4$, the solution is filtered and evaporated. The product is isolated by flash chromatography on SiO_2.

EXAMPLE 17

11,26-dihydroxyepothilone D

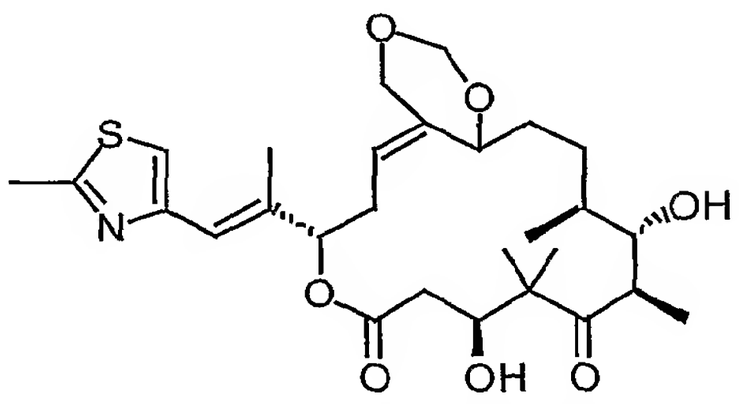


A solution of 3,7-bis(O-triethylsilyl)-11,26-dihydroxyepothilone D (see Example 6) in 2 mL of acetonitrile was treated with 50 uL of 48% aqueous HF for 24 hours. Saturated aq. $NaHCO_3$ (2 mL) was added, and the mixture was extracted with ethyl acetate. The extract was dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in 2 mL of acetonitrile, diluted with 2 mL of water, and loaded into a Varian MegaBond-Elut C18 column equilibrated in 1:1 acetonitrile/water. The column was eluted with 1:1 acetonitrile/water, and the fractions containing the product were combined and evaporated

to dryness to yield the product. An analytical sample was purified by reversed-phase HPLC using a MetaChem InertSil ODS-3 column (20 x 50 mm), eluting with a gradient from 20% to 60% acetonitrile in water at a flow rate of 8 mL/min. The product eluted at 6.1 minutes. LC/MS: $[M+H]^+ = 524$. ^{1}H-NMR ($CDCl_3$): δ 6.96 (s,1H), 6.58 (br s,1H), 5.53 (dd,1H,J=4.6,11.4 Hz), 5.21 (d,1H,J=9.6 Hz), 4.65 (m,1H), 4.43 (d,1H,J=12.0 Hz), 4.24 (br d,1H,J=11.3 Hz), 4.06 (d,1H,J=12.0 Hz), 3.70 (br s,1H), 3.16 (dq,1H,J=2.5,6.8 Hz), 2.95 (br s,1H), 2.76 (dt,1H,J=11.1,15.0 Hz), 2.69 (s,3H), 2.45 (dd,1H,J=11.3,14.9 Hz), 2.35 (dd,1H,J=4.2,15.0 Hz), 2.29 (dd,1H,J=2.6,14.9 Hz), 2.07 (d,3H,J=1.2 Hz), 1.90 (m,1H), 1.78 (m,1H), 1.6 (m,2H), 1.34 (s,3H), 1.3 (m,1H), 1.20 (d,3H,J=7.0 Hz), 1.08 (s,3H), 1.06 (d,3H,J=7.0 Hz). ^{13}C-NMR ($CDCl_3$): δ 220.3, 170.3, 165.2, 151.7, 142.1, 138.7, 126.4, 119.8, 116.0, 78.5, 74.1, 72.4, 70.4, 65.5, 53.5, 42.0, 39.5, 37.6, 33.6, 32.5, 28.6, 22.9, 19.1, 18.4, 16.1, 15.7, 13.5.

EXAMPLE 18

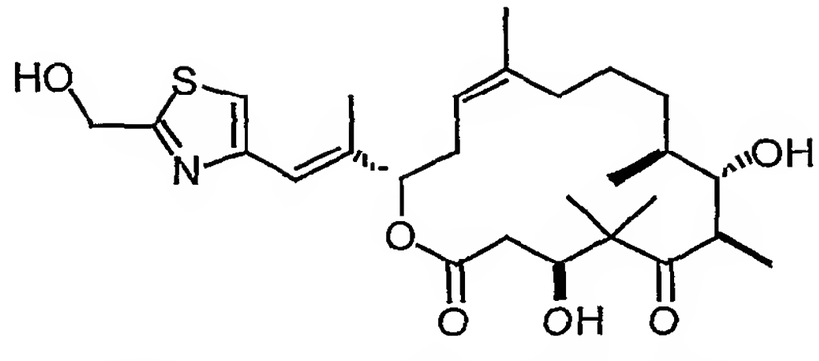
11,26-dihydroxyepothilone D formaldehyde acetal



A mixture of 11,26-dihydroxyepothilone D (Example 17, 524 mg) and pyridinium p-toluenesulfonate (50 mg) in dimethoxymethane (10 mL) is stirred for 24 hours, then treated with sat. aq. $NaHCO_3$ and extracted with ethyl acetate. The extract is washed with brine, then dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 19

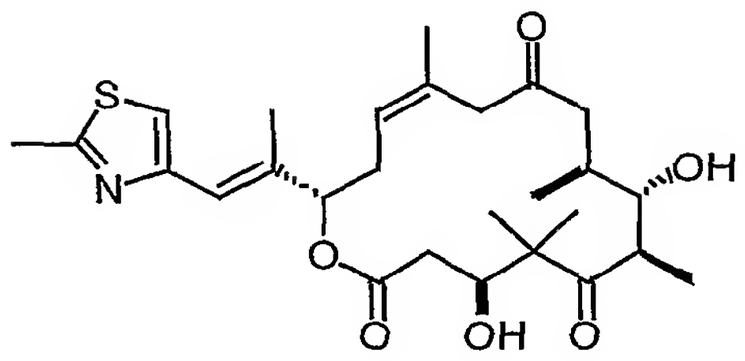
21-hydroxy-epothilone D



A culture of *Sorangium cellulosum* So ce90 *epoK*⁻ is grown at 30 °C in 8.5 liters of a medium consisting of potato starch (8 g/L), glucose (8 g/L), defatted soybean meal (2 g/L), yeast extract (2 g/L), sodium iron(III)-EDTA (8 mg/L), $MgSO_4 \cdot 7H_2O$ (1 g/L), $CaCl_2 \cdot 2H_2O$ (1 g/L), and HEPES buffer (11.5 g/L), adjusted to pH 7.4 using KOH. The culture is stirred at 150 rpm and sparged with sterile air at a rate of 0.1 volumes per minute. After 4 days of growth, the culture is concentrated to a volume of 3 liters by cross-flow filtration across a 0.3-micron membrane. A solution of epothilone D (1 g) in 10 mL of methanol is sterile filtered and added to the concentrated culture. The culture is maintained at 30 °C and is stirred at 450 rpm while sparging with sterile air at a rate of 6 liters per minute. After 24 hours, a 100-mL aliquot of XAD-16 is added to the culture and stirring is continued for an additional hour. The XAD is collected in a filter basket and washed with water to remove culture broth and cells. The XAD is then placed in a chromatography column and eluted with methanol. The eluate is concentrated to an aqueous slurry and then extracted with ethyl acetate. The extract is dried over Na_2SO_4, filtered, and evaporated to yield the crude epothilones. The 21-hydroxyepothilone D is isolated by silica gel chromatography (1:2 hexanes/ethyl acetate).

EXAMPLE 20

10-oxo-epothilone D



Step 1. 3,7-bis(O-triethylsilyl)-11-oxoepothilone D

A mixture of 11-oxoepothilone D (5.0 g) in CH_2Cl_2 (50 mL), chlorotriethylsilane (5.0 mL), and 4-dimethylaminopyridine (3.6 g) is stirred 12 hours at ambient temperature. The mixture is diluted with ether (250 mL) and washed successively with water, 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The residue is dissolved in hexanes and chromatographed on SiO_2 (hexanes/ether) to yield the product.

Step 2. 3,7-bis(O-triethylsilyl)-11-oxo-10-(phenylsulfenyl)epothilone D

A solution of 3,7-bis(O-triethylsilyl)-11-oxo-epothilone D (7.34 g) in 100 mL of THF is added dropwise to a solution of lithium diisopropylamide (10 mL of a 1.0 M solution in THF) in 100 mL of THF at –78 °C. After stirring for 1 hour, a solution of phenyldisulfide (30 g) in 50 mL of THF is added dropwise, the reaction is continued for an additional 1 hour at –78 °C, and then is allowed to warm to ambient temperature. Saturated aq. NH_4Cl is added, and the mixture is concentrated to a slurry which is partitioned between ether and water. The organic phase is washed sequentially with water, 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is chromatographed on SiO_2 (hexanes/ether) to yield the product.

Step 3. 3,7-bis(O-triethylsilyl)-11-hydroxy-10-(phenylsulfenyl)epothilone D

A solution of 3,7-bis(O-triethylsilyl)-11-oxo-10-(phenylsulfenyl)epothilone D (8.42 g) in 100 mL of THF is cooled to 0 °C and treated with a 0.5 M solution of 9-

borabicyclo[3.3.1]nonane in THF (20 mL). The mixture is allowed to warm to ambient temperature and is monitored by thin-layer chromatography. Upon consumption of starting material, the reaction is quenched by addition of water and concentrated. The residue is dissolved in ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

Step 4. 3,7-bis(O-triethylsilyl)-11-O-methanesulfonyl-10-(phenylsulfenyl)epothilone D

A solution of 3,7-bis(O-triethylsilyl)-11-hydroxy-10-(phenylsulfenyl)epothilone D (8.40 g) in 100 mL of pyridine is cooled on ice and treated with methanesulfonic anhydride (17.4 g). The mixture is warmed to ambient temperature and kept for 16 hours, then evaporated. The residue is dissolved in ether and washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine. The solution is dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

Step 5. 3,7-bis(O-triethylsilyl)-10,11-dehydro-10-(phenylsulfonyl)epothilone D

A solution of 3,7-bis(O-triethylsilyl)-11-O-methanesulfonyl-10-(phenylsulfenyl)epothilone D (9.22 g) in 100 mL of acetone is treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (15.2 g) at ambient temperature for 24 hours. The mixture is evaporated, then redissolved in ether and washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine. The solution is dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

Step 6. 3,7-bis(O-triethylsilyl)-10-oxoepothilone D

A solution of 3,7-bis(O-triethylsilyl)-10,11-dehydro-10-(phenylsulfonyl)epothilone D (8.26 g) in 100 mL of acetonitrile is treated with a solution of mercuric chloride (25 g) in 50 mL of water at ambient temperature for 24 hours. The mixture is evaporated, and the residue is dissolved in ether and washed sequentially with 1 N HCl, sat. $NaHCO_3$, and

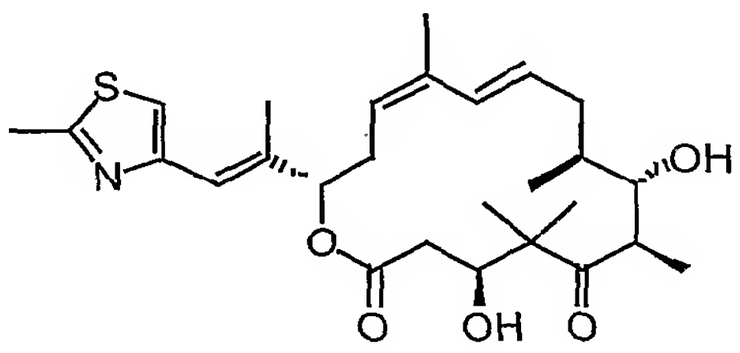
brine. The solution is dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

Step 7. 10-oxoepothilone D

3,7-bis(O-triethylsilyl)-10-oxoepothilone D (0.734 g) is added slowly to a mixture of trifluoroacetic acid (20 mL) and CH_2Cl_2 (80 mL) cooled to –15 °C. The mixture is warmed to 0 °C and stirred for 2 hours, then concentrated. The residue is chromatographed on SiO_2 to yield the product.

EXAMPLE 21

10,11-dehydro-epothilone D



Step 1. 11-(4-toluenesulfonyloxy)-epothilone D

A solution of 11-hydroxy-epothilone D (500 mg) in 10 mL of pyridine and 50 mL of CH_2Cl_2 is cooled on ice and treated with 4-(dimethylamino)pyridine (12 mg) and tosyl chloride (200 mg). After stirring for 4 hours, the mixture is diluted with ethyl acetate and washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

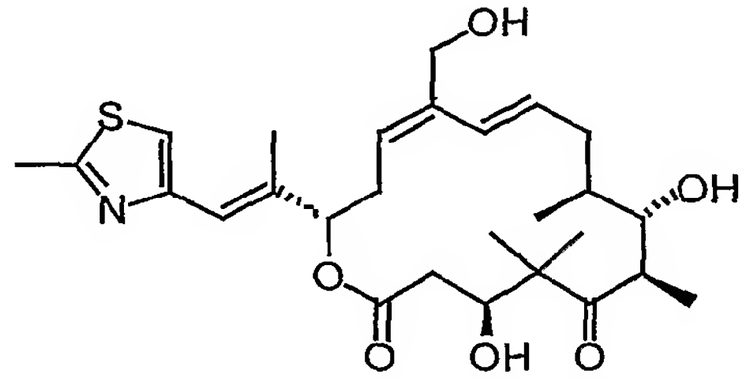
Step 2. 10,11-dehydro-epothilone D

A solution of 11-(4-toluenesulfonyloxy)-epothilone D (890 mg) in 10 mL of pyridine is treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (200 mg). The mixture is heated to 90 °C and the reaction is monitored by thin-layer chromatography. When the

starting material has disappeared, the mixture is cooled to ambient temperature and concentrated. The residue is dissolved in ethyl acetate and washed sequentially with 1 N HCl, sat. $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 22

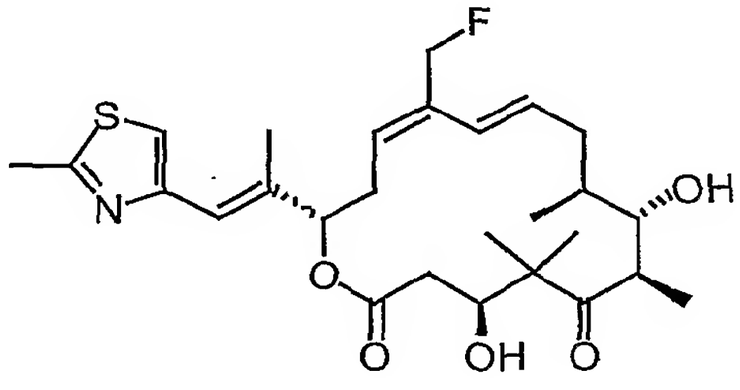
10,11-dehydro-26-hydroxy-epothilone D



A mixture of selenium dioxide (50 mg), 0.4 mL of *tert*-butylhydroperoxide (5-6 M solution in decane), 0.5 mL of dichloromethane, and 0.35 mL of water is stirred at room temperature for 15 minutes. A solution of 10,11-dehydroepothilone D (200 mg) in 1.5 mL of CH_2Cl_2 is added and the mixture is stirred for 48 hours. The mixture is diluted with 20 mL of CH_2Cl_2 and shaken with 10 mL of sat. aq. $NaHCO_3$. The phases are separated, and the organic phase is dried over $MgSO_4$, filtered, and evaporated. The residue is dissolved in 2 mL of CH_2Cl_2 and chromatographed on a 35-gm ISCO silica column to yield the product.

EXAMPLE 23

10,11-dehydro-26-fluoroepothilone D



Step 1. Preparation of 10,11-dehydro-26-iodoepothilone D

A solution of 10,11-dehydro-26-hydroxyepothilone D (5.0 g) in 25 mL of acetonitrile and 50 mL of ether is treated with imidazole (2.1 g), triphenylphosphine (7.9 g), and iodine (7.6 g). After 1 hour, the mixture is diluted with 200 mL of ether, washed with sat. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The iodide is isolated by flash chromatography on SiO_2, and is used immediately in the next step.

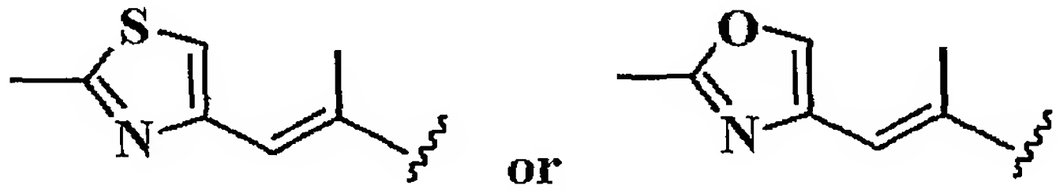
Step 2. Preparation of 10,11-dehydro-26-fluoroepothilone D

A solution of 10,11-dehydro-26-iodoepothilone D (615 mg) in 10 mL of acetonitrile is treated with a 1.0 M solution of anhydrous tetrabutylammonium fluoride in THF (2 mL) at ambient temperature for 1 hour. The mixture is diluted with 200 mL of ethyl acetate, washed with sat. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and evaporated. The product is isolated by silica gel chromatography.

EXAMPLE 24

Microbial Transformation of C-21 Methyl to C-21 Hydroxymethyl

This example describes the microbial transformation of C-21 methyl to C-21 hydroxymethyl of compounds of formula I where Ar is



A frozen vial (approximately 2 ml) of *Amycolata autotrophica* ATCC 35203 or *Actinomyces* sp. strain PTA-XXX as described by PCT Publication No. WO 00/39276 is used to inoculate 1 500 ml flask containing 100 mL of medium. The vegetative medium consists of 10 g of dextrose, 10 g of malt extract, 10 g of yeast extract, and 1 g of peptone in liter of deionized water. The vegetative culture is incubated for three days at 28°C on a rotary shaker operating at 250 rpm. One mL of the resulting culture is added to each of

sixty-two 500 mL flasks containing the transformation medium which as the same composition as the vegetative medium. The cultures are incubated at 28°C and 250 rpm for 24 hours. A suitable compound of the invention is dissolved in 155 ml of ethanol and the solution is distributed to the sixty-two flasks. The flasks are then returned to the shaker and incubated for an additional 43 hours at 28°C and 250 rpm. The reaction culture is then processed to recover 21-hydroxy counterpart of the starting compound.

EXAMPLE 25

Epoxidation using EpoK

This example describes the enzymatic epoxidation of compounds of formula I where R^8 and R^9 together form a carbon-carbon double bond (desoxy compounds of the invention). The *epoK* gene product was expressed in *E. coli* as a fusion protein with a polyhistidine tag (his tag) and purified as described by PCT publication, WO 00/31247 which is incorporated herein by reference. The reaction consists of 50 mM Tris (pH7.5), 21 μM spinach ferredoxin, 0.132 units of spinach ferredoxin: $NADP^+$ oxidoreductase, 0.8 units of glucose-6-phosphate dehydrogenase, 1.4 mM NADP, and 7.1 mM glucose-6-phosphate, 100 μM or 200 μM desoxy compound of the present invention, and 1.7 μM amino terminal histidine tagged EpoK or 1.6 μM carboxy terminal histidine tagged EpoK in a 100 μL volume. The reactions are incubated at 30°C for 67 minutes and stopped by heating at 90°C for 2 minutes. The insoluble material is removed by centrifugation, and 50 μL of the supernatant containing the desired product is analyzed by LC/MS.

EXAMPLE 26

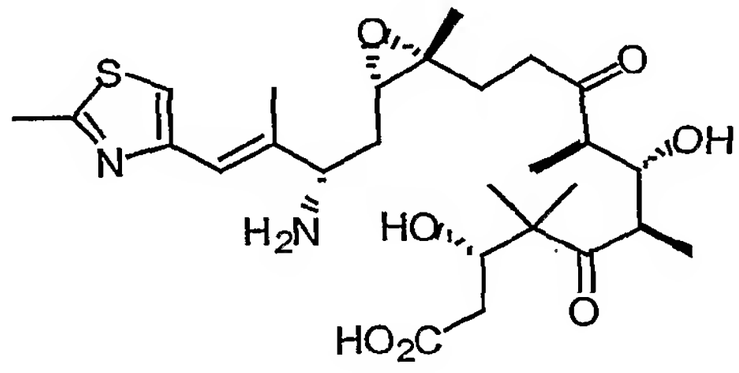
Chemical Epoxidation

This example describes the chemical epoxidation of a compound of formula I where R^8 and R^{10} together form a carbon-carbon double bond (desoxy compound of the invention). A solution of dimethyldioxirane (0.1 M in acetone, 17 mL) is added dropwise to a solution of a desoxy compound of the invention (505 mg) in 10 mL of CH_2Cl_2 at –78 °C. The mixture is warmed to –50 °C, kept for 1 hour, and then another portion of

dimethyldioxirane solution (5 mL) is added and the reaction is continued for an additional 1.5 hour at –50 °C. The reaction is then dried under a stream of N_2 at –50 °C. The product is purified by flash chromatography on SiO_2.

EXAMPLE 27

(3S,6R,7S,8R,12R,13S,15S,16E)-15-amino-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid



Step 1. 9-oxoepothilone B.

A solution of dimethyldioxirane (0.1 M in acetone, 17 mL) is added dropwise to a solution of 9-oxoepothilone D (505 mg) in 10 mL of CH_2Cl_2 at –78 °C. The mixture is warmed to –50 °C, kept for 1 hour, and then another portion of dimethyldioxirane solution (5 mL) is added and the reaction is continued for an additional 1.5 hour at –50 °C. The reaction is then dried under a stream of N_2 at –50 °C. The product is purified by flash chromatography on SiO_2.

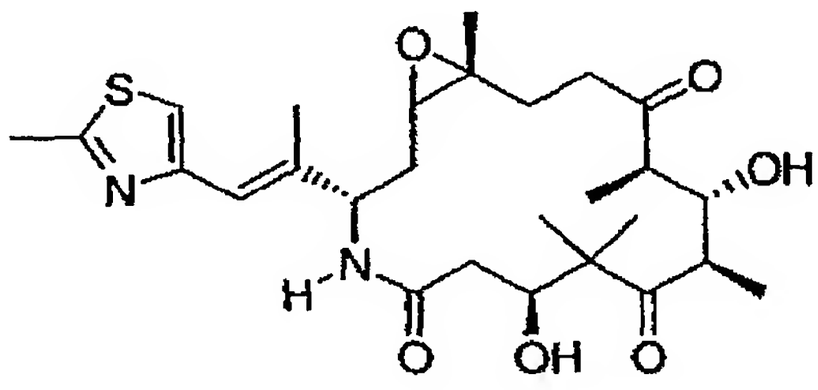
Step 2. *(3S,6R,7S,8R,12R,13S,15S,16E)*-15-azido-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid.

A solution of 9-oxoepothilone B (2.62 g) and sodium azide (0.49 g) in 55 mL of degassed tetrahydrofuran/water (10:1 v/v) is treated with tetrakis(triphenylphosphine)palladium (0.58 g) under an argon atmosphere. The mixture is kept at 45 °C for 1 hour, then diluted with 50 mL of water and extracted with ethyl acetate. The extract is washed with brine, dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

Step 3. (*3S,6R,7S,8R,12R,13S,15S,16E*)-15-amino-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid.

A solution of (*3S,6R,7S,8R,12R,13S,15S,16E*)-15-azido-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid (565 mg) in 15 mL of THF/water (10:1 v/v) is treated with a 1.0 M solution of trimethylphosphine in toluene (3 mL) under argon for 2 hours at ambient temperature. The mixture is concentrated, and the product is purified by flash chromatography on SiO_2.

EXAMPLE 28

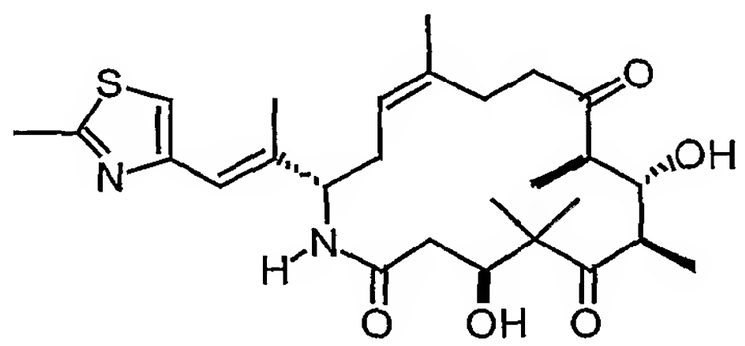


(*4S,7R,8S,9R,13R,14S,16S*)-13,14-epoxy-4,8-dihydroxy-2,6,10-trioxo-5,5,7,9,13-pentamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene.

A solution of (*3S,6R,7S,8R,12R,13S,15S,16E*)-15-amino-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid (540 mg) in acetonitrile/dimethylformamide (20:1 v/v, 150 mL) is cooled to 0 °C and treated sequentially with 1-hydroxybenzotriazole (0.135 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.5 g). The mixture is warmed to ambient temperature and kept for 12 hours, then diluted with water and extracted with ethyl acetate. The extract is washed sequentially with water, sat. $NaHCO_3$, and brine, then dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

EXAMPLE 29

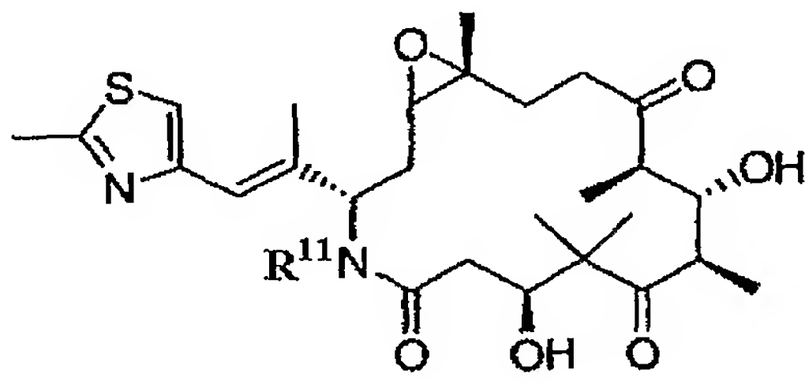
(4S,7R,8S,9R,13Z,16S)-4,8-dihydroxy-2,6,10-trioxo-5,5,7,9,13-pentamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene



A solution of tungsten hexachloride (0.76 g) in tetrahydrofuran (20 mL) at –78 °C is treated with a 1.6 M solution of n-butyllithium in hexane (2.5 mL). The mixture is allowed to warm to ambient temperature over 20 minutes. A 13.8 mL portion of the resulting green solution is added to a solution of *(4S,7R,8S,9R,13R,14S,16S)*-4,8-dihydroxy-13,14-epoxy-2,6,10-trioxo-5,5,7,9,13-pentamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene (360 mg) in 2 mL of tetrahydrofuran at ambient temperature. After 30 min, the reaction is cooled to 0 °C and treated with sat. $NaHCO_3$ (10 mL). The mixture is diluted with water and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

EXAMPLE 30

(4S,7R,8S,9R,13R,14S,16S)-13,14-epoxy-4,8-dihydroxy-2,6,10-trioxo-1,5,5,7,9,13-hexamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene.



Step 1. (*3S,6R,7S,8R,12R,13S,15S,16E*)- 3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-15-(methylamino)-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid.

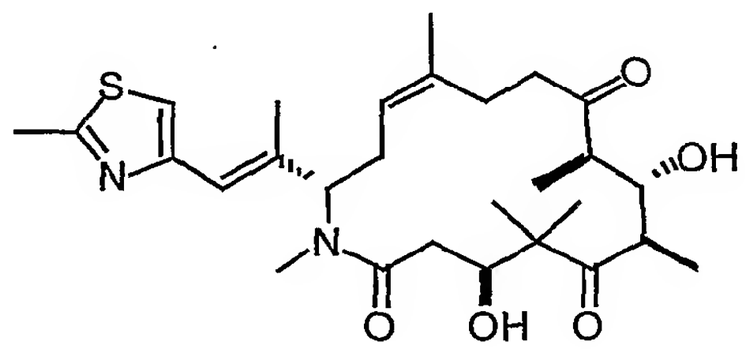
A solution of (*3S,6R,7S,8R,12R,13S,15S,16E*)-15-amino-3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid (540 mg) in 10 mL of methanol is treated with 37% aqueous formaldehyde (1 mL), acetic acid (25 uL), and sodium cyanoborohydride (100 mg). After 1 hour, then mixture is treated with 1N HCl then diluted with ethyl acetate and water. The aqueous phase is extracted with ethyl acetate, and the organic phases are combined, dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

Step 2. (*4S,7R,8S,9R,13R,14S,16S*)-13,14-epoxy-4,8-dihydroxy-2,6,10-trioxo-1,5,5,7,9,13-hexamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene.

A solution of (*3S,6R,7S,8R,12R,13S,15S,16E*)- 3,7-dihydroxy-5,9-dioxo-12,13-epoxy-4,4,6,8,12,16-hexamethyl-15-(methylamino)-17-(2-methylthiazol-4-yl)-16-heptadecenoic acid (554 mg) in acetonitrile/dimethylformamide (20:1 v/v, 150 mL) is cooled to 0 °C and treated sequentially with 1-hydroxybenzotriazole (0.135 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.5 g). The mixture is warmed to ambient temperature and kept for 12 hours, then diluted with water and extracted with ethyl acetate. The extract is washed sequentially with water, sat. $NaHCO_3$, and brine, then dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

EXAMPLE 31

(4S,7R,8S,9R,13Z,16S)-4,8-dihydroxy-2,6,10-trioxo-1,5,5,7,9,13-hexamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene



A solution of tungsten hexachloride (0.76 g) in tetrahydrofuran (20 mL) at –78 °C is treated with a 1.6 M solution of n-butyllithium in hexane (2.5 mL). The mixture is allowed to warm to ambient temperature over 20 minutes. A 13.8 mL portion of the resulting green solution is added to a solution of *(4S,7R,8S,9R,13R,14S,16S)*-13,14-epoxy-4,8-dihydroxy-2,6,10-trioxo-1,5,5,7,9,13-hexamethyl-16-(1-(2-methylthiazol-4-yl)propen-2-yl)-1-aza-11-cyclohexadecene (370 mg) in 2 mL of tetrahydrofuran at ambient temperature. After 30 min, the reaction is cooled to 0 °C and treated with sat. $NaHCO_3$ (10 mL). The mixture is diluted with water and extracted with CH_2Cl_2. The extract is dried over Na_2SO_4, filtered, and evaporated. The product is purified by flash chromatography on SiO_2.

EXAMPLE 32

Liposomal Composition

This example describes liposomal compositions containing 9-oxo epothilone. A mixture of lipids and 9-oxo-epothilone D are dissolved in ethanol and the solution is dried as a thin film by rotation under reduced pressure. The resultant lipid film is hydrated by addition of the aqueous phase and the particle size of the epothilone-derivative containing liposomes is adjusted to the desired range. Preferably, the mean particle diameter is less than 10 microns, preferably from about 0.5 to about 4 microns. The particle size may be reduced to the desired level, for example, by using mills (e.g., air-jet mill, ball mill, or vibrator mill), microprecipitation, spray-drying, lyophillization, high-pressure homogenization, recrystrytallization from supercritical media, or by extruding an aqueous

suspension of the liposomes through a series of membranes (e.g., polycarbonate membranes) having a selected uniform pore size. In one embodiment, the liposomal composition comprises: an inventive compound (1.00 mg); phosphatidylcholine (16.25 mg); cholesterol (3.75 mg); polyethyleneglycol derivatized distearyl phosphatidylethanolamine (5.00 mg); lactose (80.00 mg); citric acid (4.20 mg); tartaric acid (6.00 mg); NaOH (5.44 mg); water (up to 1 mL). In another embodiment, the liposomal composition comprises: an inventive compound (1.00 mg); phosphatidylcholine (19.80 mg); cholesterol (3.75 mg); distearyl phosphatidylcholine (1.45 mg); lactose (80.00 mg); citric acid (4.20 mg); tartaric acid (6.00 mg); NaOH (5.44 mg); water (up to 1 mL). In yet another embodiment, the liposomal composition comprises: an inventive compound (1.00 mg); 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (17.50 mg); 1-palmitoyl-2-oleyl-sn-glycero-3-phosphoglycerol, Na (7.50 mg); lactose (80.mg); citric acid (4.20 mg); tartaric acid (6.00 mg); NaOH (5.44 mg); water (up to 1 mL). Liposomal compositions containing other compounds of the present invention are prepared using conditions similar to those described above.

EXAMPLE 33

This example describes the preparation of a poly-glutamic acid-21-hydroxy-9-oxo-epothilone D conjugate. Poly(1-glutamic acid) ("PG") sodium salt (MW 34 K, Sigma, 0.35 g) is dissolved in water. The pH of the queous solution is adjusted to 2 using 0.2 M HCl. The precipitate is collected, dialyzed against distilled water, and lyophilized to yile 0.29 g of PG. To a solution of PG (75 mg, repeating unit FW 170, 0.44 mmol) in dry DMF (1.5 mL) is added 20 mg of 21-hydroxy-9-oxo-epothilone D, 15 mg of dicyclohexylcarbodiimide ("DCC") and a trace amount of dimethylaminopyridine. The reaction is allowed to proceed at room temperature for four hours or until completed as indicated by thin layer chromatography. The reaction mixture is poured into chloroform and the resulting precipitate is collected and dried in a vacuum to yield approximately 65 mg of PG-21-hydroxy-9-oxo-epothilone D conjugate. Changing the weight ratio of inventive compound to PG in the starting materials results in polymeric conjugates of various concentrations of 21-hydroxyl-10, 11-dehydroepothilone D. Conjugates of other

compounds of the present invention are prepared using conditions similar to those described above.

EXAMPLE 34

Conjugation of Epothilone D to an Antibody

Step 1. Preparation of a semicarbazone-linked epothilone

A mixture of 26-oxo-epothilone D (1 mmol), the desired semicarbazide linker, e.g., 4-(4-(2-pyridyldithio)phenyl)amino)-4-oxo-butyrate semicarbazide, (1 mmol), and sodium acetate (150 mg) in 10 mL of 1:1 ethanol/water is stirred at ambient temperature for 1 hour. The mixture is then concentrated and extracted with ethyl acetate. The extract is washed with brine, then dried over $MgSO_4$, filtered, and evaporated to give a crude 26-semicarbazone-linked epothilone D.

Step 2. Conjugation of a semicarbazone-linked epothilone to an antibody.

A solution of the antibody (at least 1 mg/mL, 1 mL) in buffer (pH 8-10) containing 5 mM dithiothreitol is kept at 37 oC for 1 hour. The solution is concentrated using ultrafiltration, diluted with thiol-free buffer, and reconcentrated. The reduced antibody solution in 1 mL of buffer is then treated with 25 uL of a 1 M methanolic solution of the pyridyldithio-semicarbazone-linked epothilone D for 1 hour at 37 oC to induce disulfide exchange. The antibody-epothilone conjugate is isolated by gel permeation chromatography.

EXAMPLE 35

Intravenous Formulaion

This example describes an intravenous formuation of 9-oxo-epothilone D. The formulation contains 10 mg/mL of 9-oxo-epothilone D in a vehicle containing 30%

propylene glycol, 20% Creomophor EL, and 50% ethanol. The vehicle is prepared by measuring ethanol (591.8 g) to a beaker containing a stir bar; adding Creomophor EL (315.0 g) to the solution and mixing for ten minutes; and then adding propylene glycol (466.2 g) to the solution and mixing for another ten minutes. 9-oxo-epothilone D (1 g) is added to a 1 L volumetric flask containing 400-600 mL of the vehicle and mixed for five minutes. After 10, 11-dehydroepothilone D is in solution, the volume is brought to 1 L; allowed to mix for another ten minutes; and filtered through a 0.22 μm Millipore Millipak filter. The resulting solution is used to aseptically fill sterile 5 mL vials using a metered peristaltic pump to a targeted fill volume of 5.15 mL/vial. The filled vials are immediately stoppered and crimped.

The vial containing 10 mg/mL of 9-oxo-epothilone D is diluted in normal saline or 5% dextrose solution for administration to patients and administered in non-PVC, non-DEHP bags and administration sets. The product is infused over a one to six hour period to deliver the desired dose.

In one embodiment, the formulation is diluted twenty fold in sterile saline prior to intravenous infusion. The final infusion concentration is 0.5 mg/mL of the inventive compound, 1.5% propylene glycol, 1 % Cremophor EL, and 2.5 % ethanol which is infused over a one to six hour period to deliver the desired dose.

Intravenous formulations containing other compounds of the present invention may be prepared and used in a similar manner.

EXAMPLE 36

Pretreatment for Cremophor® Toxicity

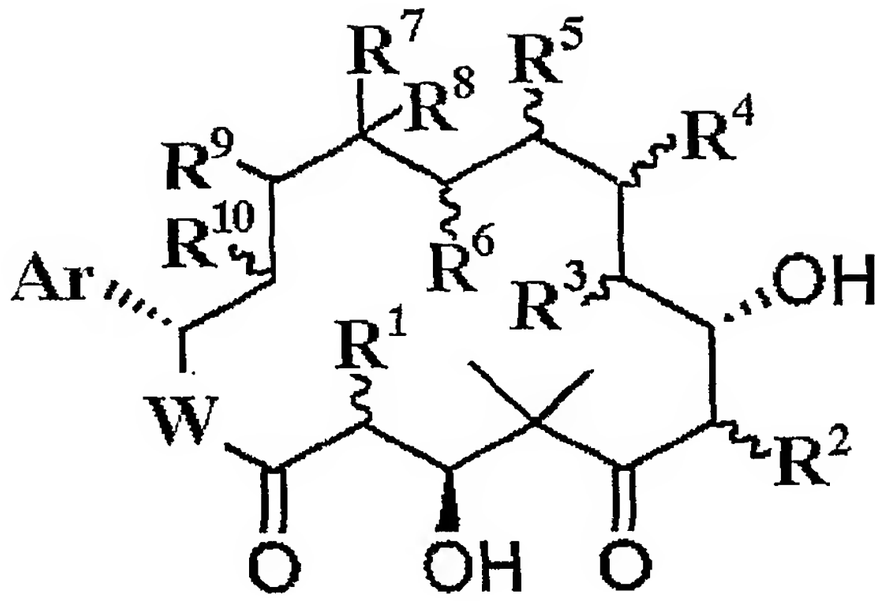
This example describes a pretreatment regiment for Cremophor® toxicity. Formulations of a compound of the invention that includes Cremophor® may cause toxicity in patients. Pretreatment with steroids can be used to prevent anaphylaxis. Any suitable corticosterioid or combination of corticosteroid with H_1 antagonists and/or H_2 antagonists may be used. In one embodiment, a subject is premedicated with an oral dose

of 50 mg of diphenylhydramine and 300 mg of cimetidine one hour prior to treatment with the inventive compound in a Cremophor® containing formulation. In another embodiment, the subject is premedicated with an intravenous administration of 20 mg of dexamethasone at least one half hour prior to treatment with the inventive compound in a Cremophor® containing formulation. In another embodiment, the subject is premedicated with an intravenous administration of 50 mg of diphenylhydramine, 300 mg of cimetidine and 20 mg of dexamethasone at least one half hour prior to treatment with the inventive compound in a Cremophor® containing formulation. In yet another embodiment, the weight of the subject is taken into account and the subject is pretreated with an administration of diphenylhydramine (5 mg/kg, i.v.); cimetidine (5 mg/kg, i.v).; and dexamethasone (1 mg/kg, i.m.) at least one half hour prior to the treatment with the inventive compound in a Cremophor® containing formulation.

All scientific and patent publications referenced herein are hereby incorporated by reference. The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments, that the foregoing description and example is for purposes of illustration and not limitation of the following claims.

What is claimed is:

1. A compound of the formula



wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl;

R^5 is hydrogen, oxo, or C_1-C_{10} aliphatic, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_{10} aliphatic, C_1-C_{10} alkylester, or halide;

R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen, or optionally, R^6 and R^7 together form a 1, 3-dioxane that is optionally substituted at the 2-position;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

Ar is aryl; and,

W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl.

2. The compound as in claim 1 where at least one of R^4, R^5 and R^6 is not hydrogen.

3. The compound as in claim 1 where R^6 is hydroxyl.

4. The compound as in claim 1 wherein R^6 is oxo.

5. The compound as in claim 1 wherein R^5 is oxo.

6. The compound as in claim 2 where

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_5 alkyl;

R^5 is hydrogen, oxo, or C_1-C_5 alkyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

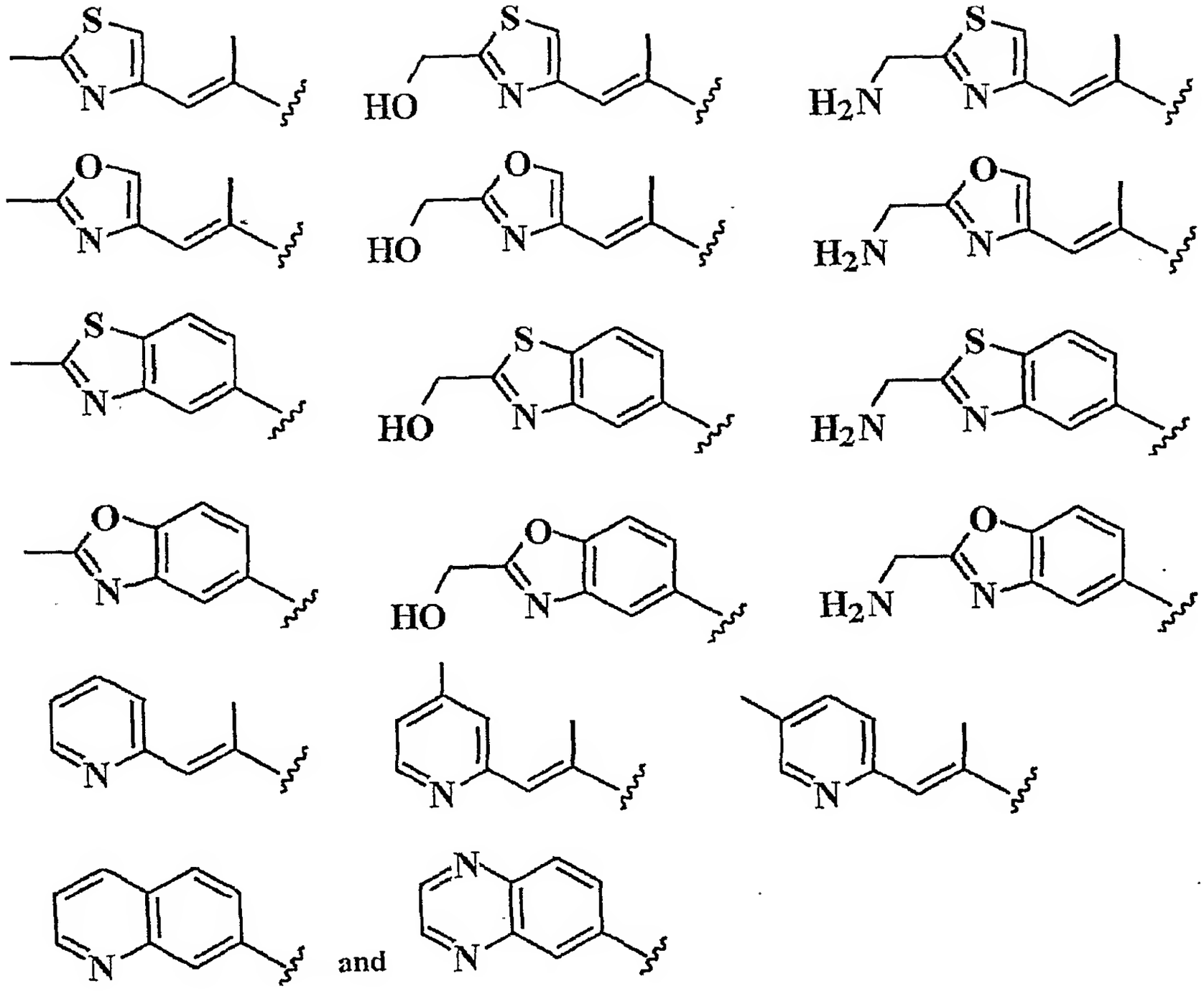
R^6 is hydrogen, hydroxyl, oxo, C_1-C_5 alkyl, or halide;

R^7 is hydrogen or C_1-C_5 alkyl that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

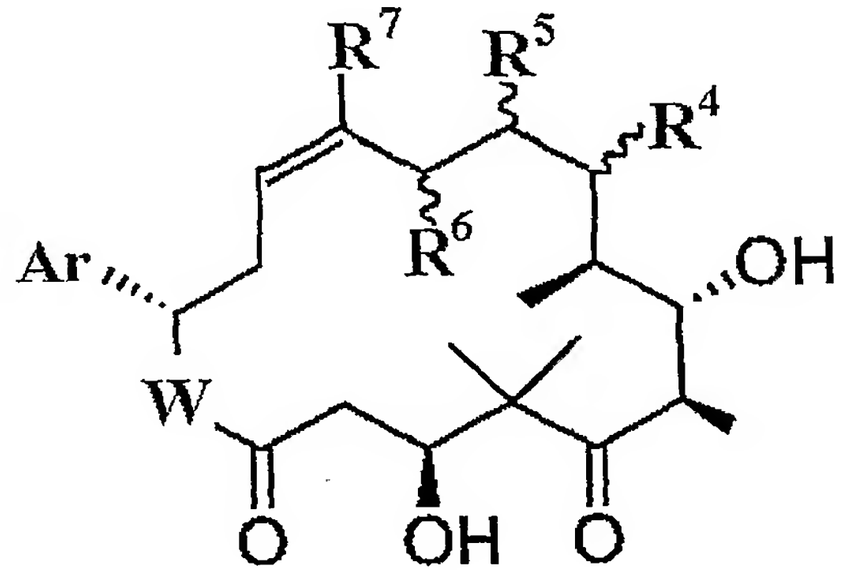
W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl; and,

Ar is selected from the group consisting of



and

7. The compound as in claim 1 of the formula



wherein:

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_5 alkyl;

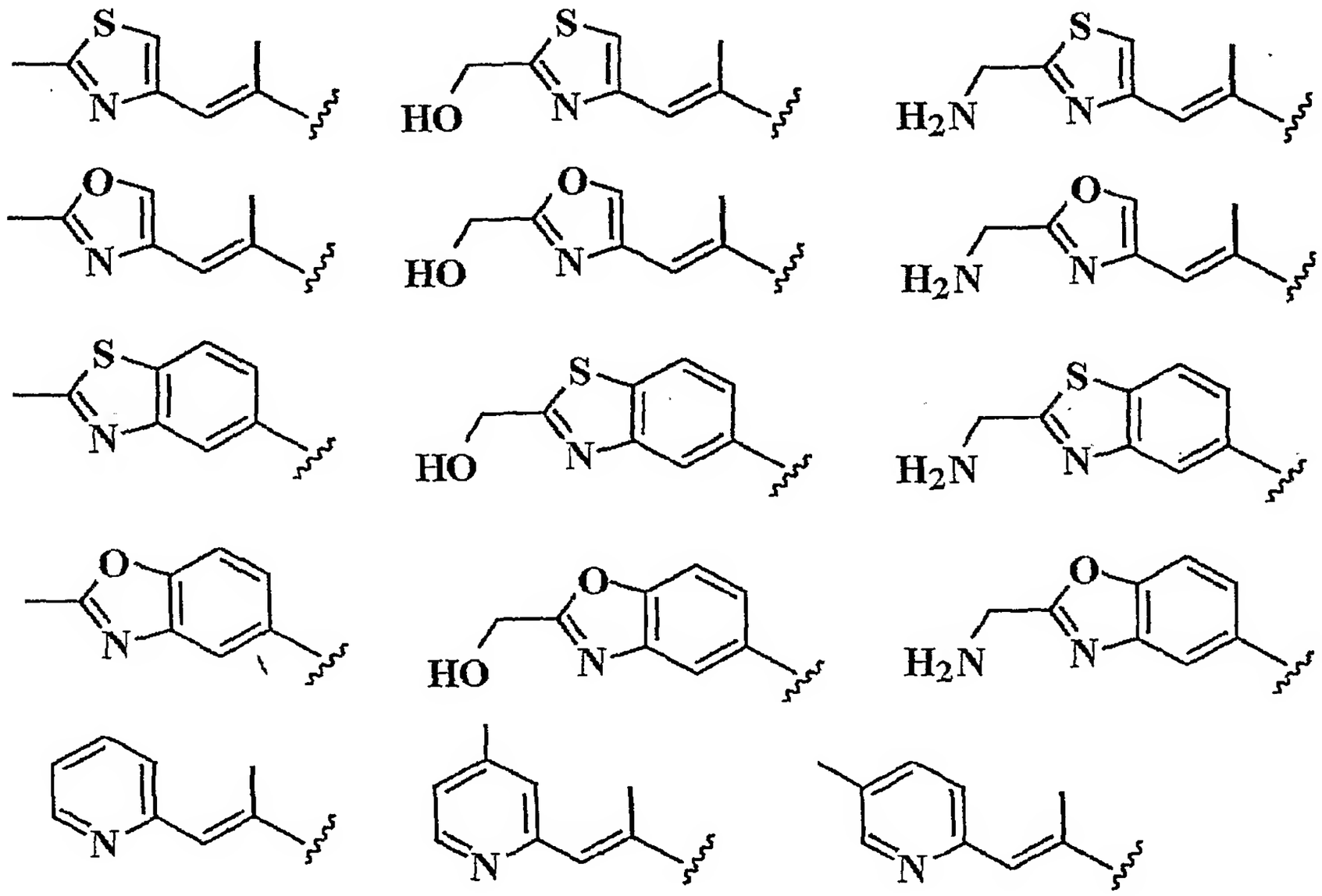
R^5 is hydrogen, oxo, or C_1-C_5 alkyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_5 alkyl, or halide;

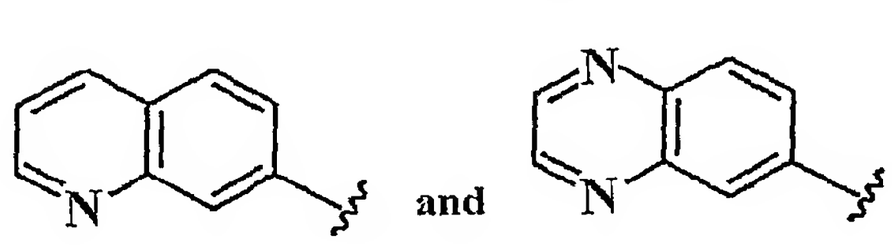
R^7 is hydrogen or C_1-C_5 alkyl that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen;

W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl; and,

Ar is selected from the group consisting of



and



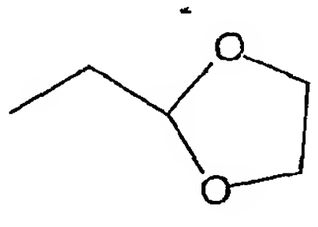
provided that at least one of R^5 or R^6 is hydroxyl or oxo.

8. The compound as in claim 7 wherein

R^4 is hydrogen or NRR' where R and R' are independently hydrogen or methyl;

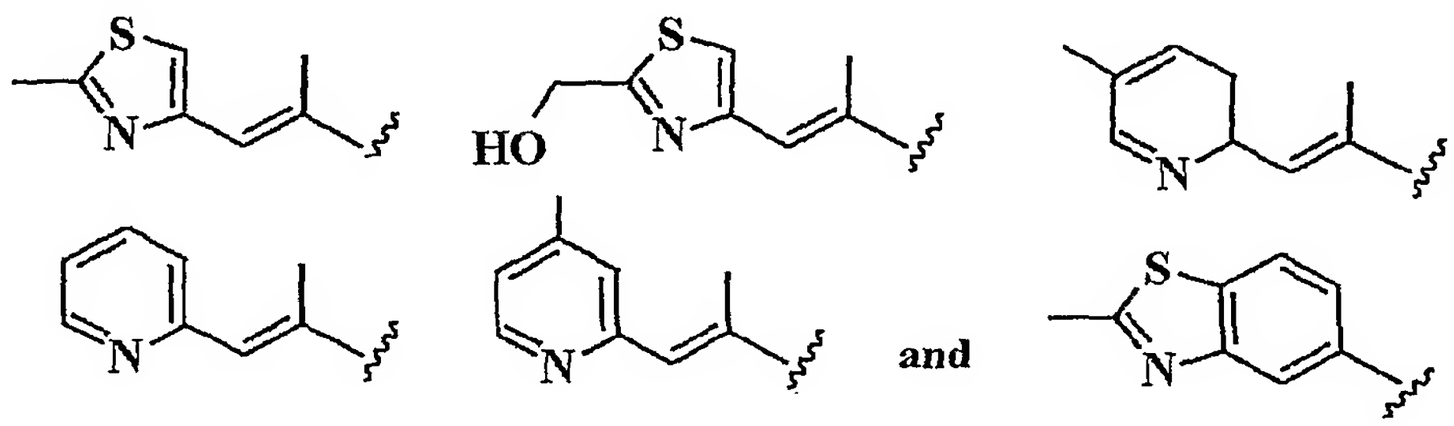
R^5 is hydrogen, oxo, or methyl, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, methyl, or halide;

R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, trifluoromethyl, $-CH_2CHO$, or  , or optionally, R^6 and R^7 together form a 1,3-dioxane;

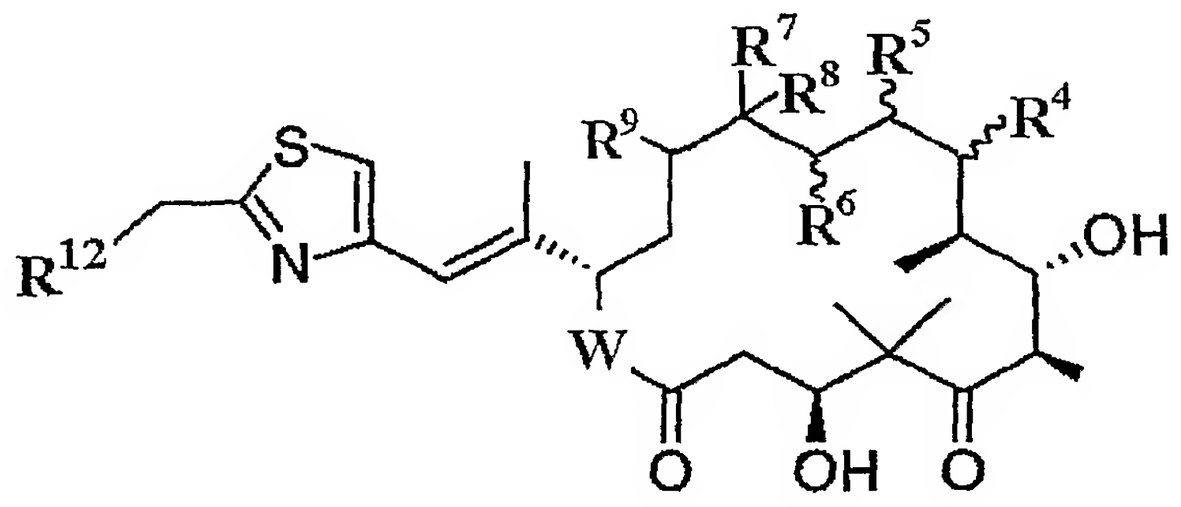
W is O or NH; and,

Ar is selected from the group consisting of



and

9. A compound of the formula



wherein:

R^4 is hydrogen, oxo, or NRR' where R and R' are independently hydrogen or C_1-C_5 alkyl;

R^5 is hydrogen, oxo, C_1-C_5 alkyl, or optionally, R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_5 alkyl or halide;

R^7 is hydrogen or C_1-C_5 alkyl optionally substituted with alcohol, aldehyde, amine dioxalane, halide, or methoxy, or optionally R^6 and R^7 together form a 1,3-dioxane;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

R^{12} is hydrogen, hydroxyl, or halide; and

W is O or NR^{11} where R^{11} is hydrogen or C_1-C_5 alkyl.

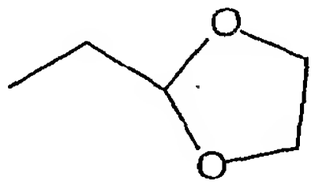
provided that at least one of R^4, R^5 and R^6 is not hydrogen.

10. The compound as in claim 9 wherein

R^4 is hydrogen, or NRR' where R and R' are independently hydrogen or methyl;

R^5 is hydrogen, oxo, or methyl, or optionally, R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, methyl or fluoro;

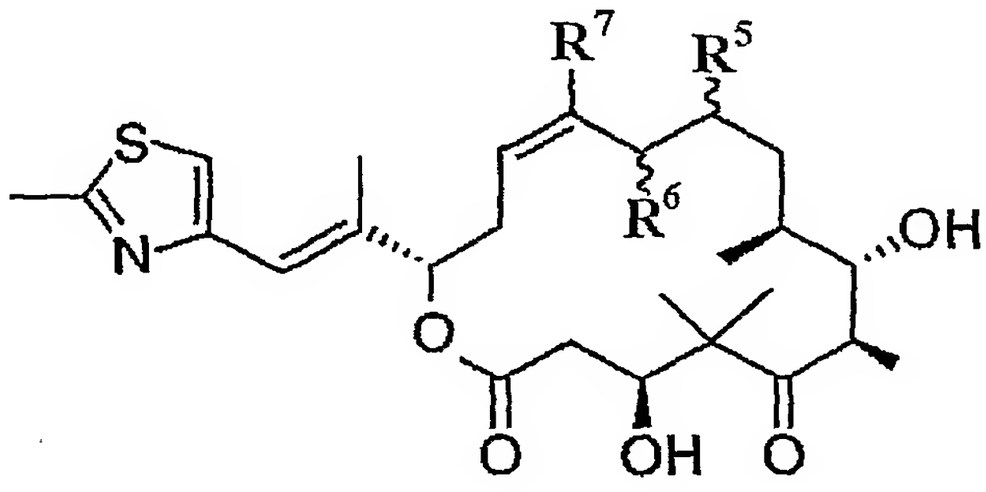
R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl, trifluoromethyl, $-CH_2CHO$, or  , or optionally, R^6 and R^7 together form a 1,3-dioxane;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

R^{12} is hydrogen, hydroxyl, or halide; and,

W is O or NH.

11. The compound as in claim 9 of the formula

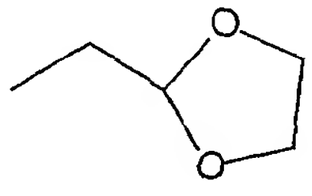


wherein

R^5 is hydrogen, oxo, or methyl;

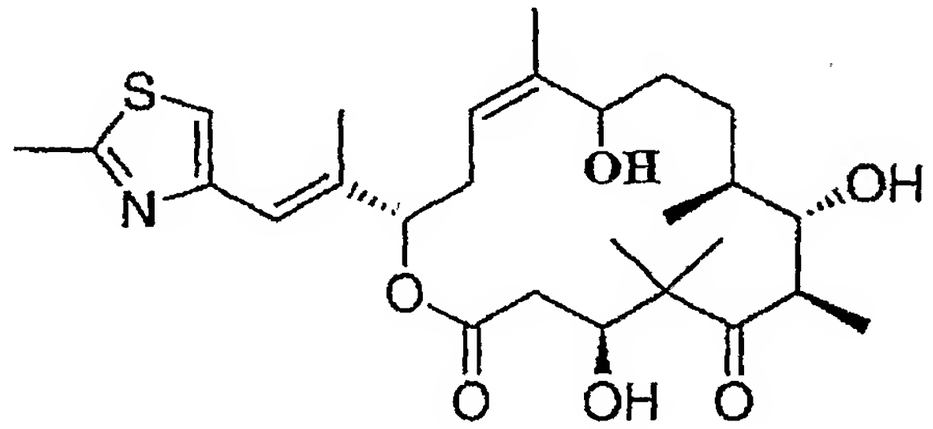
R^6 is hydrogen, hydroxyl, oxo, fluoro or methyl; and,

R^7 is hydrogen, methyl, ethyl, hydroxymethyl, fluoromethyl,

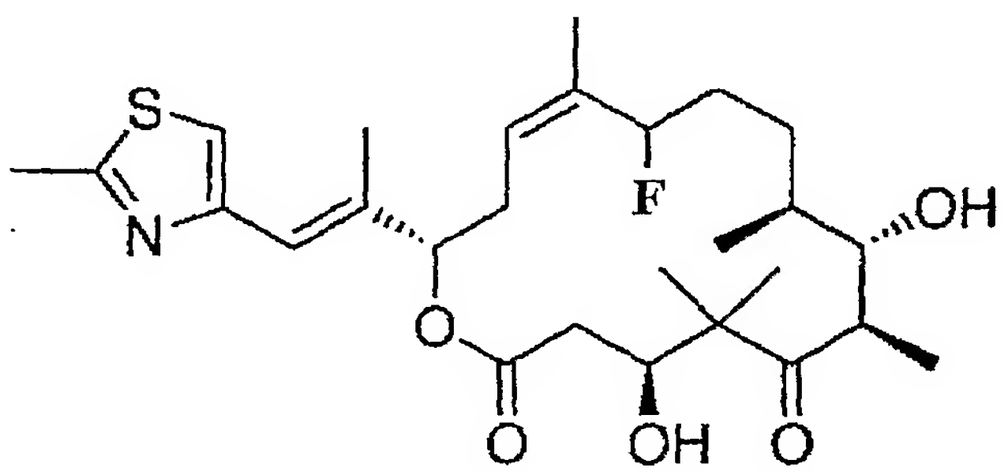


trifluoromethyl, $-CH_2CHO$, or

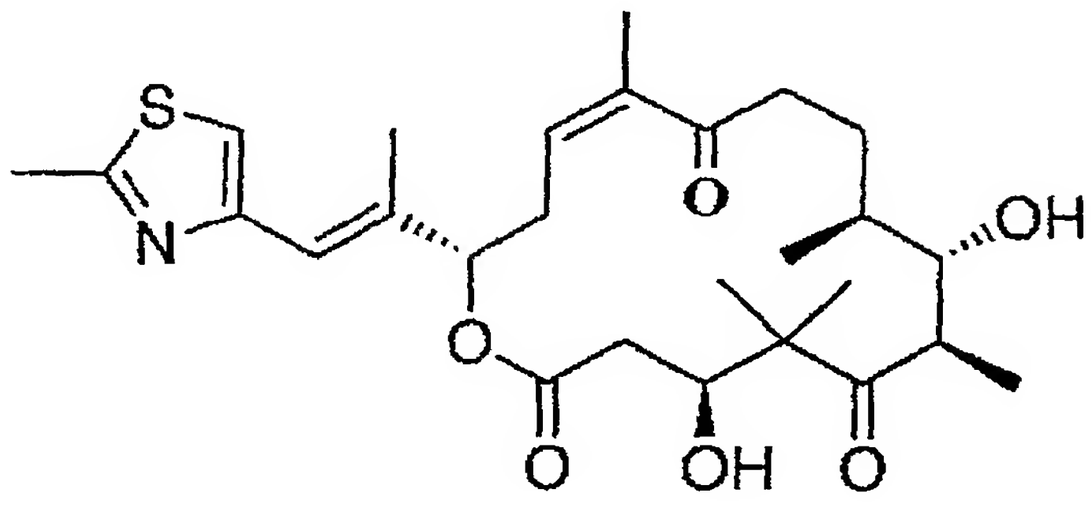
12. The compound as in claim 9 of the formula



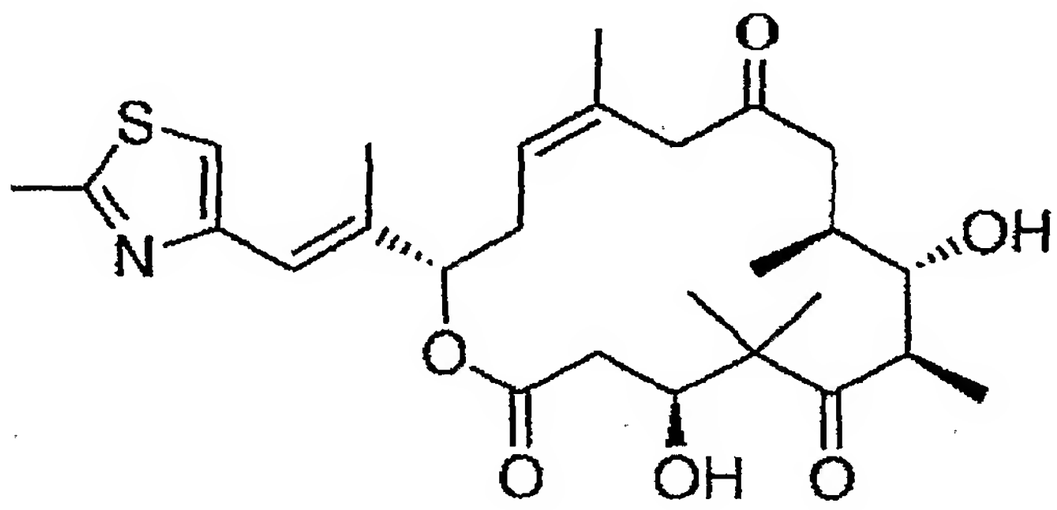
13. The compound as in claim 9 of the formula



14. The compound as in claim 9 of the formula



15. The compound as in claim 9 of the formula



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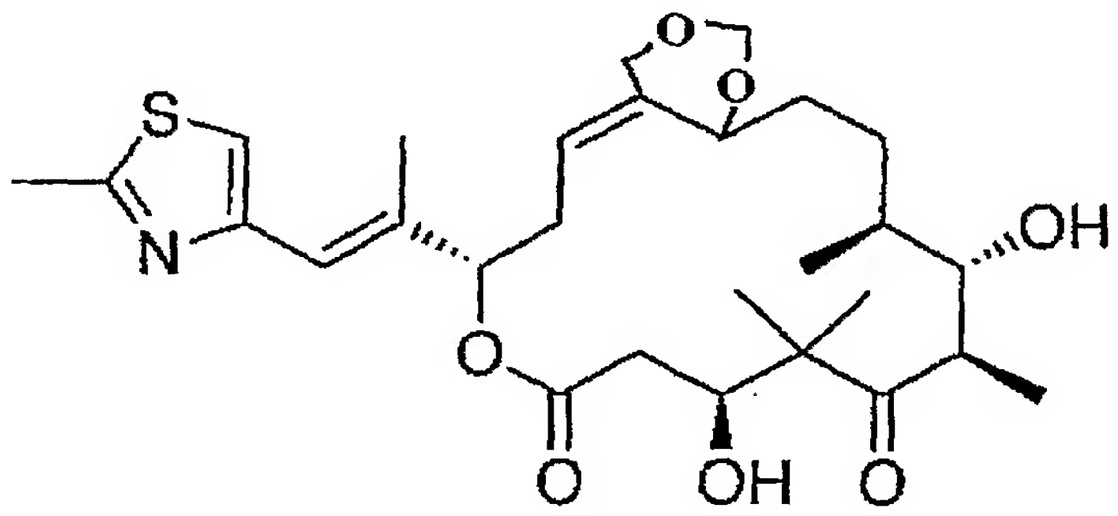
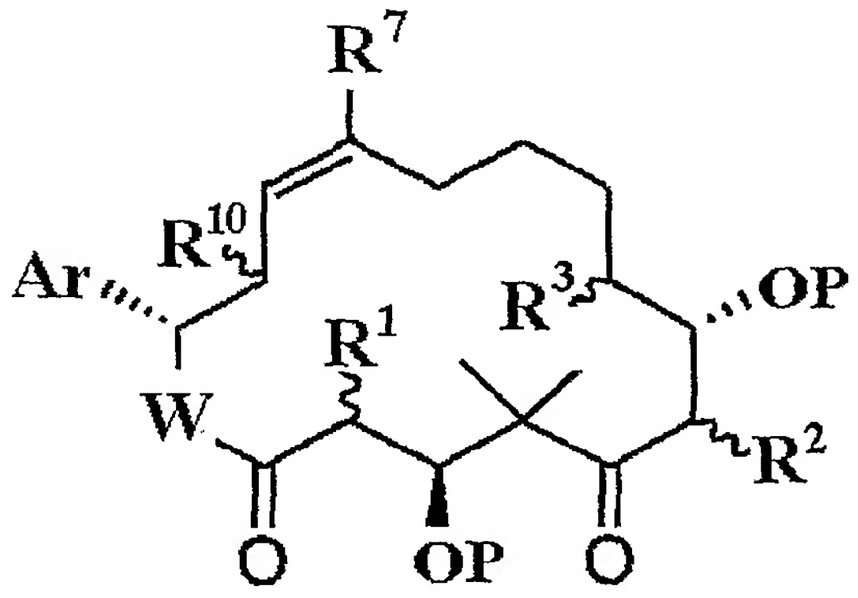
16. The compound as in claim 9 of the formula



17. A synthetic method comprising contacting a compound of the formula



with a selective hydroxylating agent wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

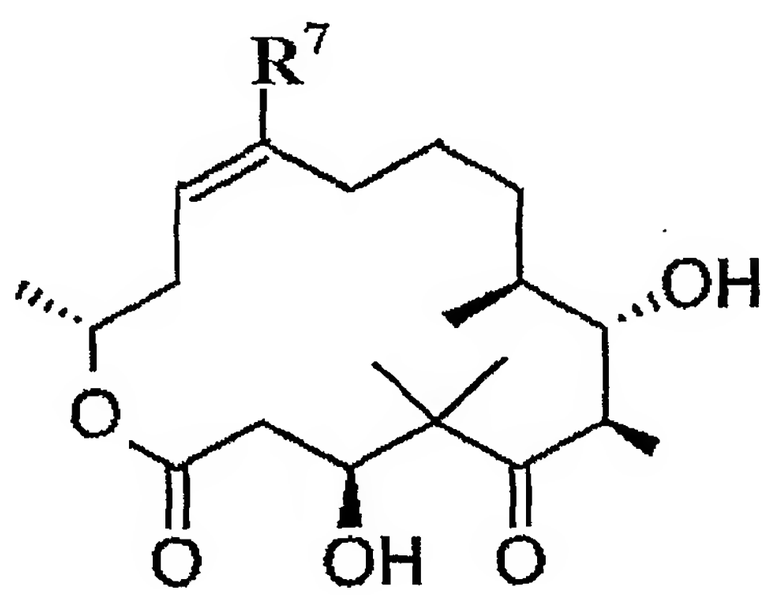
R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen;

Ar is aryl;

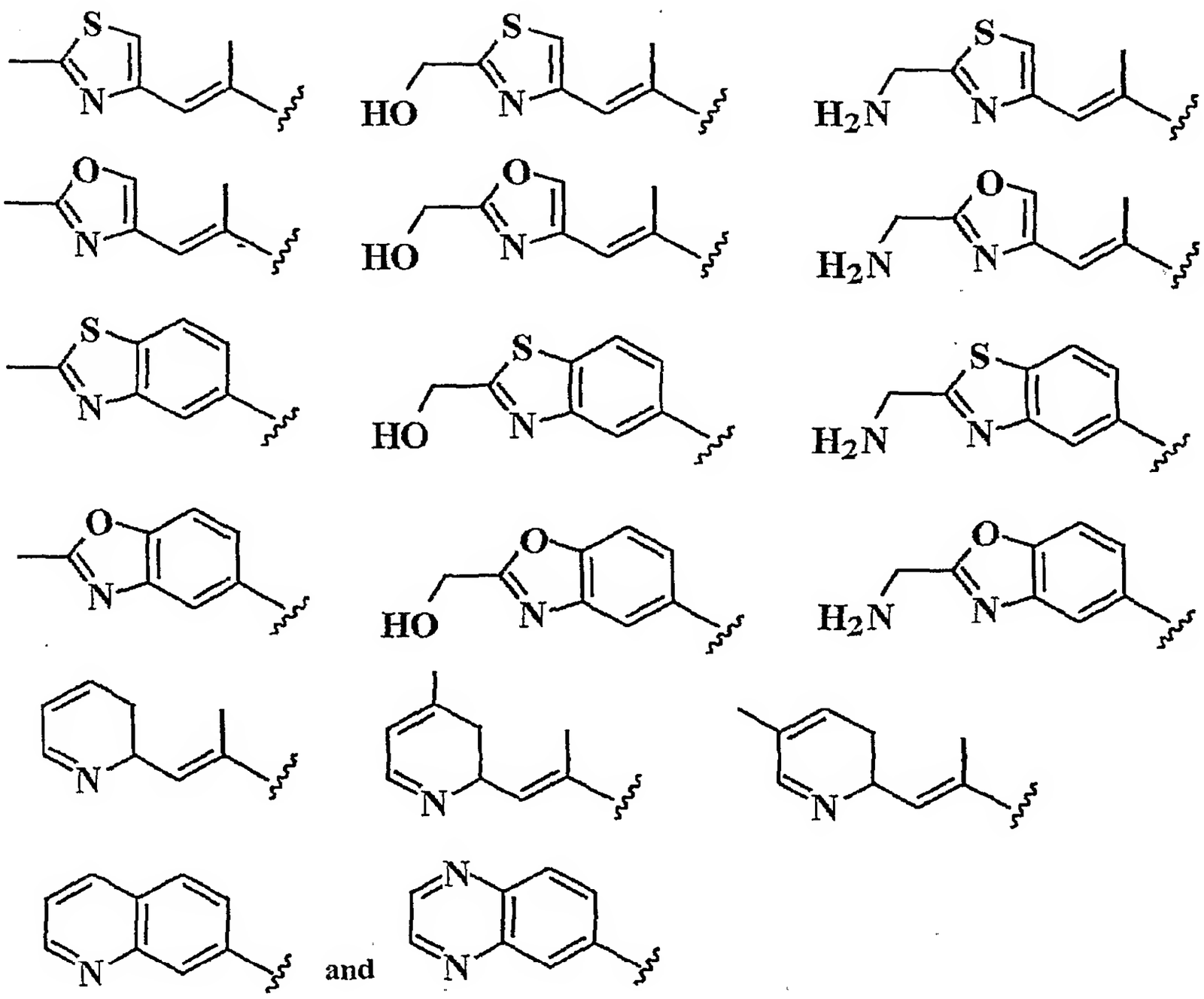
W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl; and,

P is hydrogen or a hydroxy protecting group.

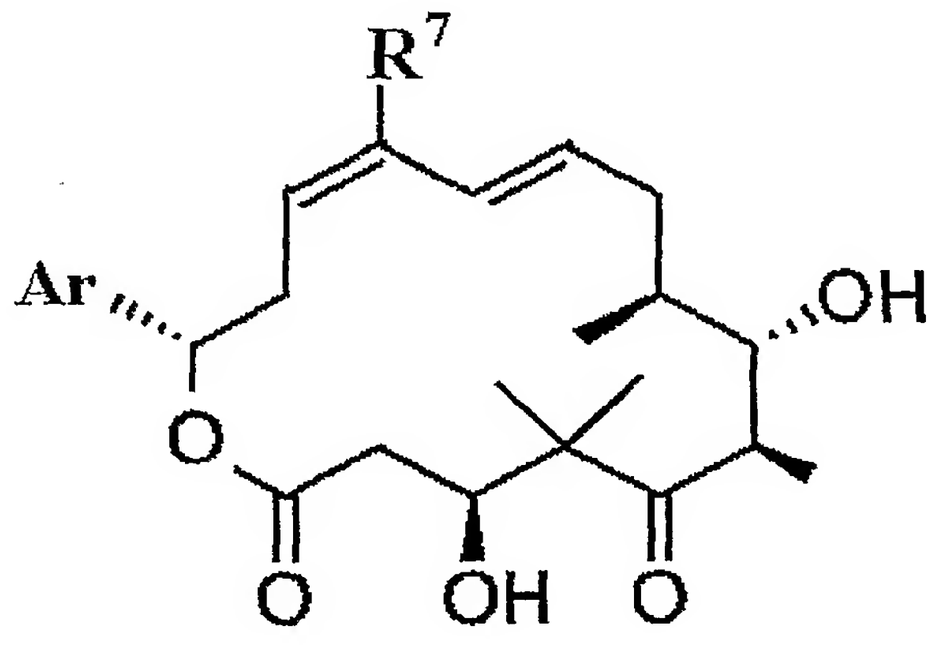
18. The method as in claim 17 comprising contacting a compound of the formula



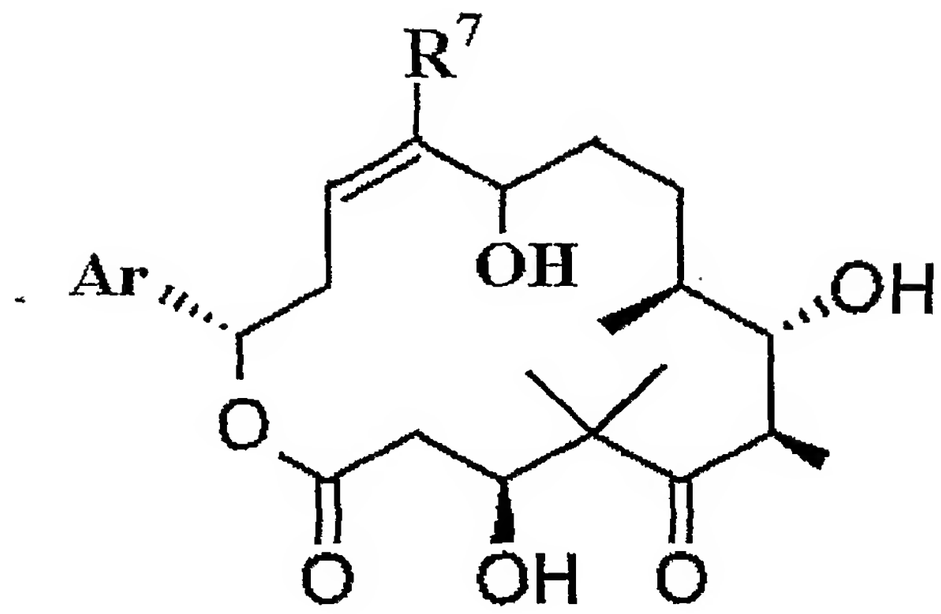
with selenium dioxide wherein R^7 is hydrogen, methyl or ethyl and Ar is selected from the group consisting of



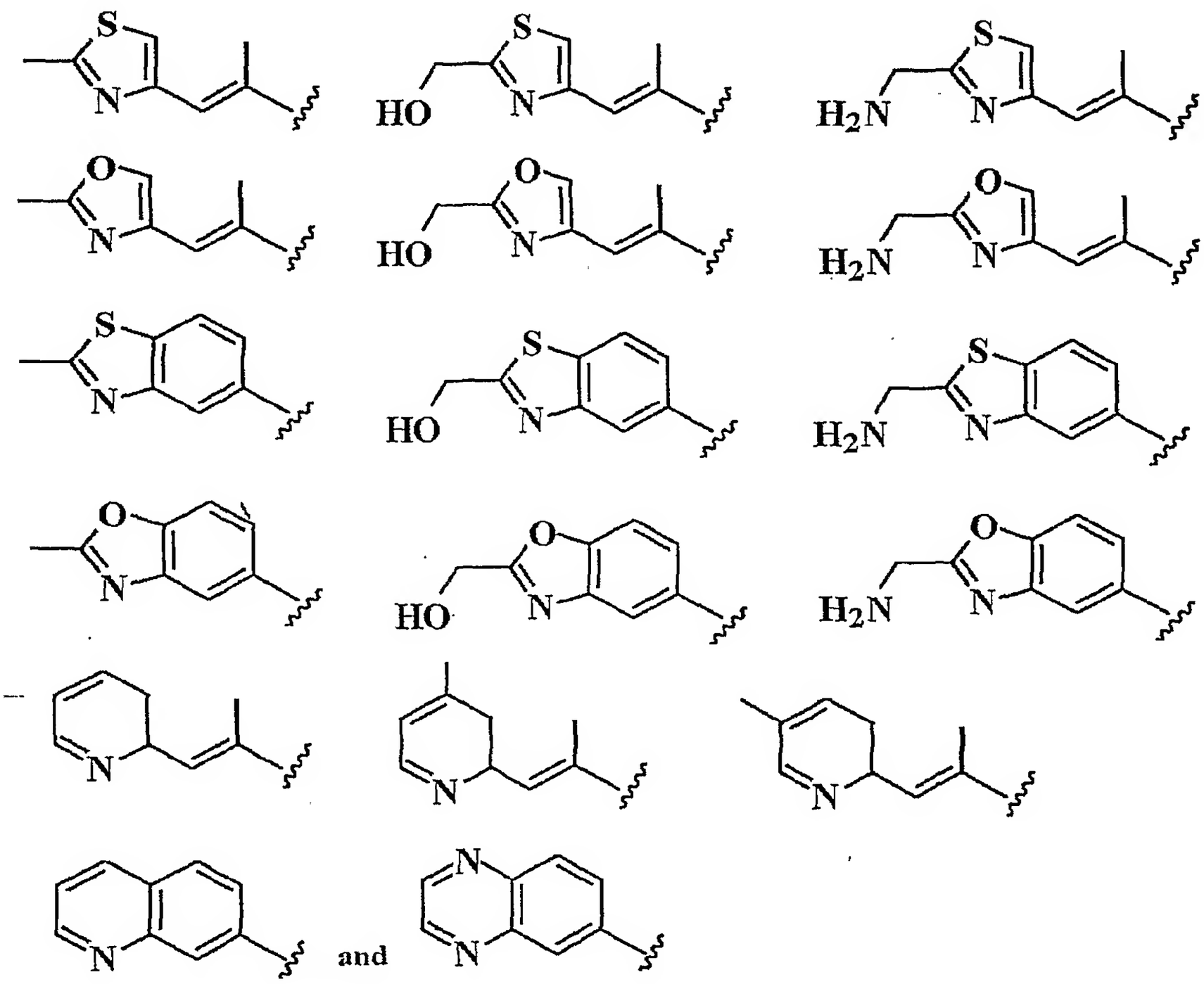
19. A dehydration method for making a compound of the formula



from a compound of the formula



where R^7 is hydrogen, methyl or ethyl and Ar is selected from the group consisting of



and

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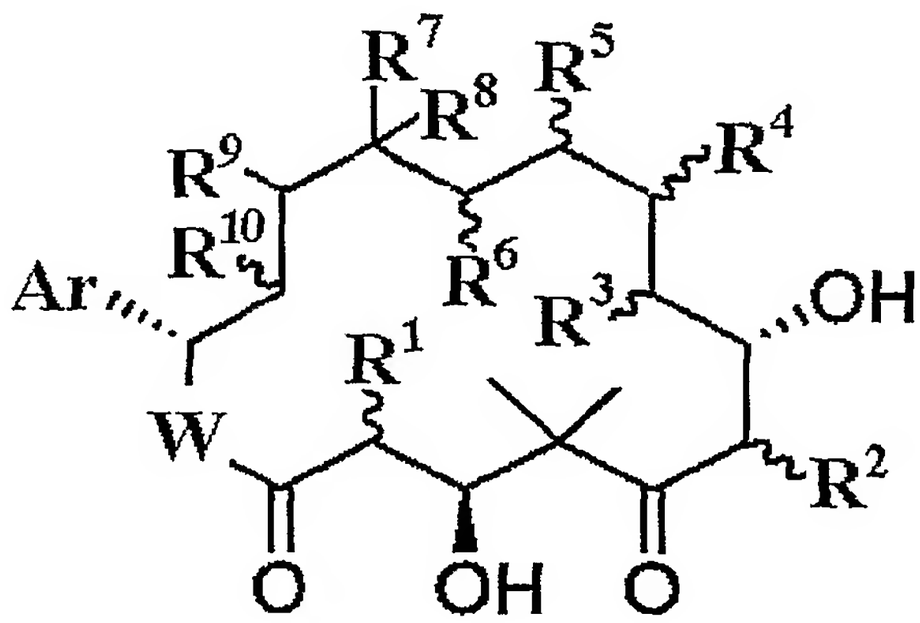
20. A method of treating a subject comprising administering a therapeutically effective amount of a compound of the formula



or a pharmaceutically acceptable salt or ester thereof wherein:

R^1, R^2, R^3, and R^{10} are each independently hydrogen, methyl or ethyl;

R^4 is hydrogen, hydroxyl, oxo, or NRR' where R and R' are independently hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl;

R^5 is hydrogen, oxo, or C_1-C_{10} aliphatic, or optionally R^4 and R^5 together form a carbon-carbon double bond;

R^6 is hydrogen, hydroxyl, oxo, C_1-C_{10} aliphatic, C_1-C_{10} alkylester, or halide;

R^7 is hydrogen or C_1-C_{10} aliphatic that is optionally substituted C_1-C_5 aliphatic, C_1-C_5 alkoxy, aryl, or a functional group selected from the group consisting of acetal, alcohol, aldehyde, amide, amine, carbamate, carboalkoxy, carbonate, carbodiimide, carboxylic acid, dioxolane and halogen, or optionally, R^6 and R^7 together form a 1, 3-dioxane that is optionally substituted at the 2-position;

R^8 and R^9 are both hydrogen or together form a carbon-carbon double bond or an epoxide;

Ar is aryl; and,

W is O or NR^{11} where R^{11} is hydrogen, C_1-C_{10} aliphatic, aryl or alkylaryl.

21. The method as in claim 20 wherein the subject has cancer.

22. The method as in claim 20 wherein the subject has a non-cancer disorder that is characterized by cellular hyperproliferation.